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RATTLESNAKE BITES IN SOUTHERN CALIFORNIA

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Snake venoms are complex mixtures, chiefly proteins, many of which have enzymatic activity. The lethal effects of these toxins are probably due to the non-enzymatic proteins, though the enzymes and enzymatic combinations certainly contribute to the over-all toxicity of the venom. In addition to the separate and combined activities of these substances, and the metabolites formed by their inter-reactions, the envenomated victim may release several autopharmacologic substances which can render diagnosis and treatment of the victim more difficult (Russell and Long[42]).

Snake venoms have been divided arbitrarily into neurotoxins, hemotoxins and cardiotoxins. While this classification has served a useful purpose, it is becoming increasingly apparent from physiopharmacological and chemical studies that neurotoxins can, and often do, have cardiotoxic or hemotoxic activity, or both; cardiotoxins may have neurotoxic or hemotoxic activity, or both, and hemotoxins may have the other activities. Until the protein fractions responsible for the deleterious effects have been isolated and studied individually, and in combination, one must consider snake venoms as substances capable of producing several changes, sometimes concomitantly, in one or more of the organ systems.

It is also apparent that quantitative, and perhaps qualitative, differences in the chemistry of rattlesnake venom may occur at the species level and may, in fact, be evident in snakes of the same species taken from different geographical locations (Goncalves[12], Schenberg[43], and Schöttler[44]). Thus, discrepancies in the mode of action of the venom and the treatment of the envenomated patient are likely to occur until our knowledge of the individual fractions of these complex substances is more complete.

Medical problem. The number of bites by venomous snakes in the United States is usually estimated to be between 1,000 and 2,000 a year (Amaral[5], Anonymous[7], Holt[16], and Stickel[52]). The author tends to agree with Hylander[17] and Bogert[9] that there are probably fewer than 1,000 such bites a year, of which approximately 70 to 75% are inflicted by rattlesnakes. The fatality figure of 3% given by Klauber[22] is reasonable. Parrish[36] lists 71 deaths due to snake bites in 5 years (1950 to

1954) of which 55 were caused by rattlesnakes.

Letters from 10 county health departments, 4 herpetologists and a number of physicians indicate that at least 52 rattlesnake bites are recorded each year in Southern California. The rattlesnake is the only venomous snake found in this area. Parrish[36] gives the average number of deaths in California from rattlesnake bites as 1.4 per year for the years 1950 to 1954. The number of deaths in Southern California attributed to these reptiles during the past 5 years is 4. Thus, the present case fatality rate for the reported cases in this area is 1.5%. This figure substantiates that proposed by Klauber[24] of probably less than 2%.

In the author's experience with 20 reptile bites, the rattlesnake was probably the offending animal in 15. In 4 cases the snake was identified as a non-poisonous reptile, and in one case the offending animal was a lizard. Identification of the species of rattlesnake involved was made in 9 of the 15 cases. The southern Pacific rattlesnake, *Crotalus viridis helleri,* was responsible for 4 of the 8 bites; the western diamondback rattlesnake, *Crotalus atrox,* was responsible for 2 bites; the sidewinder, *Crotalus cerastes cerastes,* was responsible for 2; and the Pacific rattlesnake, *Crotalus viridis oreganus,* was responsible for one. The author has had no experience with snake bites inflicted by the Colorado desert sidewinder, *Crotalus cerastes laterorepens,* the Mojave diamondback rattlesnake, *Crotalus scutulatus scutulatus,* the southwestern speckled rattlesnake, *Crotalus mitchelli pyrrhus,* and the Panamint rattlesnake, *Crotalus mitchelli stephensi.* Bites by these snakes do occur in Southern California, however (Klauber[22], and Zweifel[60]). According to Kline[25], the red diamond rattlesnake, *Crotalus ruber ruber,* is the reptile most often identified with bites to military personnel in the Camp Pendleton area. In the Palm Springs area the offending snake is usually the sidewinder, *Crotalus cerastes laterorepens* (McCammon[29]).

Adult southern Pacific rattlesnakes measure 24 to 55 inches in length, and may live for 10 to 16 years (Wright and Wright[59]). Of the 3 specimens of this species collected following bites in which they were implicated, one measured 24 inches, one 39 and one 14 inches. When 2 other specimens of 26 and 32 inches respectively were milked, 80 mg. dry weight of venom was obtained from the two glands of the smaller snake and 86 mg. from the glands of the larger one. The LD_{50}, determined intramuscularly in mice, was 7.24 mg. per kg. The intraperitoneal LD_{50} and LD_{99} for the venom of the western diamondback rattlesnake is 3.71 (3.50 to 3.92) mg. per kg. and 6.72 (6.11 to 7.39) mg. per kg. respectively, and for the eastern diamondback rattlesnake, 1.89 (1.63 to 2.19) mg. per kg. and 2.97 (2.18 to 4.08) mg. per kg. (Russell and Emery[40]). In the same experiment the intramuscular LD_{50} and LD_{99} for the venom of the latter species was 19.04 (15.61 to 23.23) mg. per kg. and 28.0 (23.26 to 31.90) mg. per kg.

On the basis of these figures, and the estimated lethal dose for the venom of the western diamondback rattlesnake (Minton[31]), the approximate lethal dose for the venom of the southern Pacific rattlesnake for a 70 kilogram man would be 53 mg. However, as pointed out by Minton[31] and Klauber[22], it may not be justifiable to calculate the lethal dose of a venom for man on the basis of data obtained from mouse toxicity tests. The author's clinical experiences and the results obtained from several experiments conducted with monkeys would seem to

indicate that the approximate lethal dose for man, for the venom of the southern Pacific rattlesnake, would be 1.0 mg. per kg. of body weight.

Klauber[23] has indicated the importance of some of the factors which affect the gravity of rattlesnake bites. The age and size of the victim, the nature, location, depth, and number of bites, the length of time the snake holds on, the extent of anger or fear that motivates the snake to strike and the amount of venom ejected, the species and size of snake involved, and the condition of his fangs and venom glands are all factors which influence the severity of the bite. In addition, the victim's sensitivity to the venom, the pathogens present in the animal's mouth and the first-aid treatment may also alter the course of the poisoning. The ability of the envenomated victim to release autopharmacologic substances may also influence the measure of the bite. It can be seen that a rattlesnake bite may vary in severity from one of little danger to one of extreme gravity.

TABLE 1.—SYMPTOMS AND SIGNS FOLLOWING RATTLESNAKE BITES IN SOUTHERN CALIFORNIA

Symptoms and Signs	*Author's Cases*	*Other Cases**
Fang marks	15/15†	33/33
Swelling and edema	15/15	33/33
Ecchymosis	11/15	26/33
Pain	13/15	30/33
Swelling regional lymph nodes	9/10	7/8
Weakness	9/15	9/19
Sweating	9/15	5/12
Increase body temperature	8/15	10/23
Faintness or dizziness	7/15	7/14
Nausea or vomiting, or both	7/15	6/16
Numbness or tingling of tongue and mouth or scalp	7/12	3/10
Decrease in hemoglobin	6/15	5/16
Hemorrhagic vesiculations	6/15	6/16
Fasciculations	5/12	2/6
Blood pressure changes	5/15	12/27
Weak pulse	5/15	5/17
Increase pulse rate	5/15	12/25
Respiratory rate changes	5/15	6/20
Increase blood clotting time	5/15	2/6
Tingling or numbness of affected part	5/15	2/12
Necrosis	4/15	6/16
Hematemesis	4/15	2/18
Leukocytosis	4/9	6/14
Respiratory difficulties	4/15	4/12
Unconsciousness	4/15	5/11
Shock	4/15	5/11
Abnormal electrocardiogram	3/14	2/6
Convulsions	0/15	1/28
Paralysis	0/14	0/13

*Contributed by Doctors C. R. Anderson, A. B. Brower, J. Carlucci, I. A. Fields, E. Gettelman, C. L. Haines, Jr., J. R. Huntsman, E. F. Kline, A. E. Martin, C. J. McCammon, N. P. Papageorges and R. G. Zweifel.

†15/15: Number of times symptom or sign was observed in total number of cases.

SYMPTOMS AND SIGNS. The symptoms and signs following bites by rattlesnakes in Southern California are summarized in Table 1. As has been previously noted, the venoms of the rattlesnakes involved in the present series of cases may differ both quantitatively and qualitatively; thus, there

are some essential differences in the findings associated with the bites of the various species of these snakes. The bite of the western diamondback rattlesnake tended to cause more pain, edema, ecchymosis, necrosis, hemorrhagic vesiculations, vomiting, hematemesis and hemolytic anemia than that of the southern Pacific rattlesnake. The latter's bite produced more marked changes in consciousness, more profuse weakness and sweating, more tingling or numbness over the affected part and tongue, mouth and scalp, and more severe respiratory difficulties.

In the author's experience, bites by the sidewinders, *Crotalus cerastes*, have tended to be relatively mild. This may have been due in part to the small size of the snake involved as the venom of this species is known to be very toxic (Minton[30]). Despite its small size the sidewinder is occasionally responsible for fatal ophidiasis in Southern California. The symptoms and findings seen following bites by the red diamond rattlesnake are similar, but less severe, than those seen following bites by the western diamondback rattlesnake. This observation appears to be confirmed by the findings of Kline[25].

Swelling and edema were the most consistent findings observed following envenomation in the present series of cases. The swelling was usually seen about the injured area within 5 minutes after the bite and progressed over the extremity during the ensuing 1 to 36 hours. In 7 of the 15 cases, the edema extended beyond the involved extremity. Ecchymoses may appear within 20 minutes following the bites of the western diamondback rattlesnake. They usually occur later following bites by the southern Pacific rattlesnake, though in one case in the present series, extensive ecchymosis was observed 20 minutes following the injury.

Pain is usually associated with rattlesnake bites. The intensity varies with the species of snake and the amount of venom involved. The pain is most severe following bites by the western diamondback rattlesnake and the red diamond rattlesnake. In 2 poisonings by the southern Pacific rattlesnake and one by the sidewinder the pain was described as only minor.

The marked weakness and sweating following poisonings by the southern Pacific rattlesnake were consistent findings. These symptoms were usually associated with a rapid, thready, weak pulse and some decrease in systemic arterial pressure and body temperature. In the more severe poisonings (Grade 2 and 3, Wood, Hoback and Green[58]), changes in sensory perception, or tingling, were frequently reported. True paralysis was not observed by the author in his series of cases; however, contractures, probably initiated as flexion reflexes stimulated by the pain were occasionally seen. The venom of the western diamondback rattlesnake is a very weak neuromuscular blocking agent (Russell and Long[42]). Muscular fasciculations were seen in some of the patients.

Laboratory findings varied considerably. Mild leukocytosis, sphering of the red blood cells and defects in coagulation were sometimes seen. In 4 patients, progressive hemolytic anemia developed with the hemoglobin eventually falling below 10.0 gm. An elevated blood urea nitrogen and serum bilirubin were found in 2 of 6 patients. Hypofibrinogenemia was seen in 3 patients, hematuria in 4, proteinuria in 2, and glucosuria in 6.

Deaths from rattlesnake bites in Southern California have been attributed to intraperitoneal or retroperitoneal hemorrhage associated with marked hemolytic anemia, acute pulmonary edema associated with respiratory failure, or vascular collapse

associated with acute hemolysis. Laboratory experiments indicate that vascular collapse may occur through the direct action of the venom on the heart (Russell and Emery[41]).

TREATMENT. According to Tenery and Koefoot[53] over 6,000 papers have been published on snake bites and venoms. It is evident that until our knowledge of the chemical and zootoxicological properties of these venoms is more complete there can be no general agreement on a standard of procedure for the treatment of rattlesnake ophidiasis. The therapeutic suggestions offered in this report are based upon the results obtained in the present series of cases, this Laboratory's experimental work on rattlesnake venom, and the opinions expressed by several physicians and investigators who have had extensive experience with this problem, namely Doctors L. M. Klauber, S. A. Minton, Jr., H. M. Parrish, C. B. Pollard and C. H. Pope.

It is difficult to evaluate the effectiveness of the various suggested first aid measures. In reviewing the many papers noted by Klauber[22] in his chapter, "Treatment and Prevention of the Bite," one finds that those victims who received incision and suction, and application of a tourniquet, had a higher survival rate than those who did not receive this treatment, though the statistical significance is questionable if one also takes into account the other important variables (deaths from one species or family of venomous snake versus deaths from a different species or family, size of snake and victim, and the like). Experiments with animals (Allen[2,3], Jackson[18], Jackson and Githens[20], Jackson and Harrison[21], Leopold, Huber and Kathan[28], Parrish[34], Pope and Peterson[39]) are sometimes difficult to interpret and in some cases not at all convincing. One must be exceedingly careful in applying data obtained from experiments on rodents, to humans. For instance, if one calculates the LD_{50} in mice for the venom of the eastern diamondback rattlesnake and applies these data to humans, he finds that it would require 6 adult rattlesnakes to inflict an LD_{50} dose into a 70 kg. man. Also, in mice and guinea pigs, if one uses cryotherapy as suggested (Stahnke[49]), he will increase the LD_{50} to the LD_{99} (Russell and Emery[41]). One wonders, even from the standpoint of certain anatomical relationships, whether the application of a loose tourniquet about the thigh of a rabbit is comparable with the application of a tourniquet to the ankle of a man. Obviously, these data must be interpreted with care.

There is good experimental and clinical evidence to indicate that immobilization of the affected part with absolute rest for the victim are warranted following a rattlesnake bite. Application of a constriction band directly above the wound site, if the wound is on an extremity, would appear to be indicated. It should be released for 90 seconds every 15 minutes. Cruciate or longitudinal incisions of one-eighth to one-quarter inches in length through the fang marks are advised in those cases where the offending rattlesnake has been a large one, or the victim a child. The direction of the animal's strike and the curvature of the fang should be borne in mind when determining the plane of the incision. The depth of fang penetration may be taken as approximately three-quarters of the distance between the two fang marks (Klauber[22]). Multiple incisions over the involved extremity or in advance of progressive edema are not advised.

Suction should be employed over the incisions during the first hour subsequent to the bite unless there is an abnormal amount of bleeding or obvious defect in coagulation. The pri-

mary purpose of incision is to expose the track through which the fang passed so that suction can be applied directly to the area in which the venom has been injected. Suction, properly applied, may remove a portion of the venom from the wound site (Jackson and Harrison[21]). The author has collected, in one hour, a sufficient amount of hemorrhagic exudate from an incised wound in a dog to kill an adult guinea pig. The symptoms and findings were similar to those observed following direct envenomation of a guinea pig. In 2 of the author's cases and in 4 cases contributed by other physicians, a large amount of serosanguineous fluid was obtained on incising the bitten area. In all of these cases, the patient reported some relief from pain, the localized edema was lessened and the attending physician described the patient's condition as "improved" following the cut. At the present time on the basis of these observations, the author feels justified to advise incision and subsequent suction for one hour following the bite by any of the rattlesnakes of Southern California.

After the first hour the affected part should be placed in an ice bath and the tourniquet removed. The ice bath should be replaced with ice bags one hour later. The bags may be retained about the injured extremity for 1 to 3 days. Forms of cryotherapy for snake bite have been advocated or discussed by Crum (1906)[10], Haw (1907)[14], Willson (1908)[56], Orndoff (1915)[33], Allen (1938)[2], Allen (1939)[3], Allen (1949)[4], and more recently by Stahnke (1954)[50]. Shannon[47,48] has indicated some of the dangers involved in using cryotherapy. Wood[57], Parrish[35], Efrati and Reif[11] and an Anonymous report[8], have advised against its use while Andrews and Pollard[6], Tenery and Koefoot[53], and Watt and Pollard[55] seem to favor it. Unfortunately, the claims made by some advocates of cryotherapy are grossly exaggerated, unsupported by critical clinical observation, and based on poorly controlled animal experimentation. Nonetheless, the measure may be of definite therapeutic value in depressing the proteolytic and other enzymatic activities of the venom. Whether this treatment has any marked effect on the non-enzymatic proteins, or lethal fractions, of the venom is not known, though it seems unlikely. However, in the present series of cases the application of ice to the involved extremity resulted in a decrease in the amount of edema, or a slowing of the rate of advancement of the edema. Quite contrary to the claim by some (Stahnke, Allen, Horan and Tenery[51]), cryotherapy has had no effect on the mortality rate from rattlesnake bites in Southern California. In 6 of the 7 deaths attributable to rattlesnakes, and known to the author, adequate cryotherapy was instituted early in the course of the treatment. In 3 of these 7 deaths, in the author's opinion, the amount of antivenin administered was not sufficient.

None of the above first aid measures should in any way be regarded as substitutes for "the three A's" (Watt, Parrish and Pollard[54]), antivenin, antibiotic and antitoxins; nor should they be instituted at the possible expense of delaying administration of the antivenin.

The importance of early antivenin therapy is well established (Klauber[22], and Schöttler[45]). The precautions and routes of administration for Wyeth, Antivenin (Crotalidae) Polyvalent are outlined in the brochure enclosed with each package of the antivenin. The amount of antivenin used in an individual case depends on the species and size of the snake involved, the size of the victim, the number of bites, and

other factors. For bites by the southern Pacific rattlesnake the author has used 3 to 7 vials in each case. Children bitten by larger rattlesnakes will require at least 4 vials and possibly 8 or 9. In these victims early administration of the antivenin cannot be overemphasized. Bites by the sidewinder require 1 to 4 vials. The route for administering the antivenin varies with the individual case. If the victim is not sensitive to the antivenin it has been our practice to divide the first vial into three portions and give one portion subcutaneously at various points around the involved extremity, above the bite, or in advance of the swelling. Antivenin should never be injected into a toe or finger, however. The second portion is given intramuscularly into a large muscle mass of the involved extremity and the last portion is administered intravenously in 5% glucose in water. Subsequent doses should be given intramuscularly or intravenously. We have found the anterior thigh to be a good area for repeated intramuscular injections of the antivenin.

Unfortunately, there is no commercially available antivenin of the potency of Wyeth's, prepared from the serum of animals other than horses. Studies in this Laboratory indicate that it may be possible to produce a more effective antivenin without using animal blood serums. If the patient is sensitive to horse serum, desensitization should be carried out as indicated in the Wyeth brochure. It may be worthwhile to use only the globulin fraction of the antivenin in markedly sensitive patients. ACTH and cortisone are of value in controlling the untoward reactions to horse serum.

Because of the large number of hemolytic anemias observed following bites by the southern Pacific rattlesnake, the author routinely types all patients on admission to the hospital. Whole blood transfusions were given in 5 of the patients in the author's series.

Antihistamines appear to be contraindicated in the treatment of Crotalidae envenomation (Lefrow and Michard[27], Parrish, Scatterday and Moore[38], and Schöttler[46]). As gas gangrene and tetanus organisms have been isolated from the mouths of snakes (Jackson[19], Parrish, MacLaurin and Tuttle[37]) it seems advisable to give the appropriate toxoids or antitoxins.

ACTH and cortisone are used rather extensively throughout the United States in the treatment of ophidiasis. There appears to be very little agreement on the rationale for using these drugs and considerable difference as to their effectiveness (Allam, Weiner and Lukens[1], Harris[13], Knapp and Flowers[26], Parrish[35], Schöttler[46], and Shannon[48]). In experiments with animals (Minton[32], and Schöttler[46]) they appear to have no effect on the lethal dose of the venom. However, Wood, Hoback and Green[58] report a definite improvement in the clinical symptoms attending the use of these drugs. Doctors H. M. Salk and C. J. Marsch, veterinarians of Palm Springs, have treated approximately 500 dogs for rattlesnake bites and feel that the use of prednisolone, with antivenin, has definitely reduced the morbidity rate and length of hospital stay.

The author has used the corticosteroids in several clinical cases but has not been able to determine whether they were of value in either reducing the severity of the symptoms or shortening the period of hospitalization. In 2 patients, both infants, discontinuing the ACTH resulted in a decrease in the patients' irritability, and improvement in consciousness level and no noticeable change in the amount of edema or other findings. In most of the deaths studied by the author, which

have been attributed to rattlesnakes, one of the corticosteroids had been employed. These drugs have had no effect on the morbidity rate of rattlesnake bites in Southern California. The author feels that the physician's reliance should be placed in the antivenin, and that until our knowledge of the mechanism of action by which the corticosteroids enter into this complex reaction is more complete, their use should be limited to combating the allergic manifestations provoked by the venom or the horse serum. If one of these substances is to be used, methylprednisolone would appear to be the drug of choice.

A number of other substances have been suggested for the treatment of ophidiasis. These include methoxamine hydrochloride, epinephrine, heparin, prostigmine, potassium permanganate, atropine, nitroglycerin, digitalis, coramine, caffeine sodium benzoate, strychnine, gold chloride, alum water, calcium chloride, india ink, ammonia, the iodides, petroleum products, alcohol, indigo, and others. Unfortunately, snake venoms are complex mixtures composed of both nonenzymatic and enzymatic proteins, and nonproteins, which produce many changes in the body and which also provoke certain autopharmacological responses. It seems unwise to advise the use of a drug for snake bite poisoning on the basis of its action as a "pressor agent," "oxidizing agent," "cerebral stimulant," and the like, unless the physician is well aware of the action of the drug on the other parameters of the organ systems.

Summary. Approximately 50 rattlesnake bites are reported each year in Southern California. The present case fatality rate is 1.5%. Of the 9 species of rattlesnakes common to the area, the majority of injuries are inflicted by the southern Pacific rattlesnake, *Crotalus viridis helleri*, the red diamond rattlesnake, *Crotalus ruber ruber*, the western diamondback rattlesnake, *Crotalus atrox*, and the sidewinder, *Crotalus cerastes laterorepens*. While symptoms and signs following rattlesnake ophidiasis may differ with the species of snake involved, edema and swelling, ecchymosis, pain, and swelling of the regional lymph nodes are consistent findings. Frequently observed are: weakness, sweating, increase in body temperature, faintness, nausea or vomiting, or both, numbness or tingling of tongue and mouth or scalp, decrease in blood hemoglobin, and hemorrhagic vesiculations. Treatment consists of immobilization of the affected part, complete inactivation of the patient, application of a tourniquet, incision and suction, ice packs, antivenin, antibiotic and antitetanus agents. Attention should be given to the maintenance of cardiovascular and respiratory tone. Repeated red blood cell counts and hemoglobin determinations are advised during the entire treatment and immediate follow-up periods.

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SUMMARIO IN INTERLINGUA

Morsuras de Crotalo in Sud-California

Approximativemente 50 morsuras de crotalo es reportate annualmente in Sud-California. Le mortalitate es currentemente 1,5 pro cento. Novem species de crotalo es commun in le area, sed le majoritate del morsuras es infligite per *Crotalus viridis helleri, C. ruber ruber, C. atrox,* e *C. cerastes laterorepens.* Durante que le signos e symptomas que seque ophidiasis crotalin differe ab un specie de crotalo al altere, edema e tumescentia, ecchymosis, dolor, e allargamento del local nodos lymphatic es notate invariabilemente. Constatationes frequente es debilitate, sudor, augmento del temperatura corporee, nausea e/o vomito, torpiditate o formication del lingua e del bucca o del corio capillute, reduction del hemoglobina in le sanguine, e vesiculationes hemorrhagic. Le tractamento consiste del immobilisation del parte interessate, complete inactivation del patiente, application de un tourniquet, incision e suction, compressas glaciate, antivenina, e agentes antibiotic e antitetanic. Attention debe esser prestate al mantenentia del tono cardiovascular e respiratori. Repetite numerationes del erythrocytos e determinationes de hemoglobina es recommendabile durante le complete periodo de tractamento e immediatemente post illo.

SUMMARIO IN INTERLINGUA

(See page 51 for original article)

Le Influentia del Erythrocytos Super le Coagulation del Sanguine

In un tubo de proba, le reaction initial in le coagulation de sanguine es le activation de un constituente plasmatic per contacto con le vitro. Le activate constituente plasmatic reage con un factor plachettal, producente un agente que pare esser identic in su activitate con le factor coagulatori erythrocytina le qual es presente in le erythrocytos.

Le these que erythrocytina es formate in le plasma e transferite in le erythrocytos es supportate per le observation que le consumption de prothrombina es basse quando sanguine total es miscite con un equal volumine de plasma ab le mesme donator e que es povre in plachettas e ha habite nulle contacto cŏn vitro ante le immixtion.

Le consumption de prothrombina in le coagulation de sanguine normal depende in parte del quantitate de erythrocytina producite in excesso del quantitate transferite in le erythrocytos.

Le erythrocytina de erythrocytos intacte es apparentemente non prestemente disponibile pro le cogaulation, sed un leve vulneration del erythrocytos pote causar su liberation. Le effecto de iste phenomeno super le tempore del consumption de prothrombina es discutite, e le effecto probabile de un massive hemolyse intravascular es signalate.

AN ANALYSIS OF THE CLINICAL APPLICATION OF THE VAN DEN BERGH REACTION IN JAUNDICE

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The relationships of the direct reacting, the indirect reacting and the total serum bilirubin have been used since the test was devised by van den Bergh and Muller[19] as a diagnostic aid in differentiating "medical" from "surgical" jaundice. With the development of newer concepts of bilirubin metabolism (Billing and Lathe[4], Schmid[26,27,28], Schmid, Hammaker and Axelrod[30]), a critical re-evaluation of the serum van den Bergh reaction as a diagnostic aid seems necessary.

Some investigators (Deenstra[10], Watson[33]) have suggested that within specific ranges the ratio of direct and total serum bilirubin may be valuable in differentiating jaundice. Specifically, Watson[33] has suggested "that the one-minute bilirubin (direct) is often elevated significantly within the normal range of total bilirubin in diseases of the liver or biliary tract. Low bilirubin ratios in cases of elevated total serum bilirubin are indicative of retention jaundice of various degrees. The lowest ratios (less than 20%) are characteristic of hemolytic disease or constitutional hepatic dysfunction of the Gilbert type. While the majority of patients with jaundice due to parenchymal liver disease have bilirubin ratios in the same general range as found in cases of biliary obstruction (45% to 80%) there is a significant segment of cases with diffuse liver disease in which ratios below 40% are encountered. In the range of 35% to 40% there is small likelihood of confusion with biliary obstruction and below 35% almost none." However, other workers (Gray[11], Gray and Whidborne[12], Hoffbauer, Rames and Meinert[18], Klatskin and Drill[21], With[34], Zieve *et al.*[37], Zinninger[38]) have not felt that the direct and indirect van den Bergh reactions were of diagnostic significance except when suspecting hemolytic anemia.

Early, it was thought that the van den Bergh reaction was governed by Beer's law in reference to the concentrations of solution (Gray[11], Gray and Whidborne[12]). Although it has been proven that bilirubin in human plasma is bound to plasma albumin (Greenspan and Dreiling[13], Klatskin and Bungards[20]), the van den Bergh reaction does not depend on splitting of the protein linkage by alcohol (Cole and Lathe[6]). Later, by chromatographic methods and analysis it was found that the direct and indirect frac-

tions contained distinct substances which could be identified as free bilirubin, bilirubin monoglucuronide, and bilirubin diglucuronide (Billing[2], Billing, Cole and Lathe[3], Billing and Lathe[4], Cole and Lathe[6], Cole, Lathe and Billing[7], Lathe[22], Schmid[28]). Accordingly, it has been suggested that the names "direct" and "indirect reacting bilirubin" be replaced by "bilrubin" and "conjugated bilirubin" (Lathe[22]).

Other workers (Arias and London[1], Grodsky and Carbone[14], Schmid[27], Schmid *et al.*[29], Schmid, Hammaker and Axelrod[30]), have given presumptive evidence of the method of enzymatic elaboration of the bilirubin glucuronides. Thus, transferase enzymes in the microsomes of liver cells, catalyze the transfer of the glucuronide radical from uridine diphosphate glucuronic acid (UDPGA) to various receptors. In this case, the receptor is bilirubin and the glucuronide is found through the carboxylic groups of bilirubin (Schachter[25]).

It has been demonstrated that there is a definite difference in solubility in water between bilirubin and its esterglucuronides (Billing, Cole and Lathe[3], Himsworth[17]). It is proposed that the solubility differences between bilirubin and its glucuronide in water and alcohol is the basis for the "indirect" and "direct" van den Bergh reaction. The more readily soluble glucuronides are normally excreted in bile (Billing, Cole and Lathe[3,4,7], Schmid[27], Schmid *et al.*[29,30]).

Many tests of serum have been advocated in an effort to differentiate extrahepatic biliary obstruction and hepatocellular jaundice: alkaline phosphate (Simmons[31]); alkaline phosphatase and thymol flocculation (Maclagan[23]); alkaline phosphatase, total cholesterol, thymol turbidity (Wang[32]); serum iron (Christian[5]); cephalin cholesterol flocculation, alkaline phosphatase, bilirubin (Hanger[15]); direct and total serum bilirubin (Watson[33]); serum alkaline phosphatase, serum glutamic transaminase serum glutamic pyruvic transaminase (Wroblewski[35,36]); intraglobulin fractional analysis (Greenspan and Dreiling[13]); pigment I and pigment II (Billing and Lathe[4]). It is clear that this differentiation might be made during the early phase of extrahepatic obstruction but not later since cholangitis and parenchymal damage eventually develop in unrelieved obstruction. The obstructive picture of elevated alkaline phosphatase and bilirubin in cholestasis due to chlorpromazine and methyl testosterone also tends to be confusing. Negative reactions of the empirical tests for hepatocellular damage and normal responses to the tests of synthetic power are valuable confirmatory evidence of the conclusions based on clinical grounds. Positive results are of little value except in early cases in attempts to differentiate the two types of jaundice (Himsworth[17]). Single sample determination has many limitations (Hoffbauer, Rames and Meinert[18]). Hanger[15] states that "nonetheless, the combination of a negative cephalin flocculation with a rising alkaline phosphatase and deepening jaundice is the most accurate indication of obstructive jaundice that has yet been devised."

Since the direct and total serum bilirubin continue to be performed by most clinical laboratories and to be used by physicians in an effort to differentiate medical and surgical jaundice, the following statistical study of cases with a well established diagnosis and elevated serum bilirubin on single sample determination was completed.

Materials and Methods. The medical records of approximately 1000 patients admitted to St. Louis City Hospital between 1953 and 1957 were reviewed. One hundred and one patients with total bilirubin above 1.2 mg.

per 100 ml. or direct above 0.2 mg. per 100 ml., or both, were carefully selected on the basis of unequivocal diagnosis established at necropsy, biopsy, exploratory laparotomy or, in a few cases, history of contact and typical clinical course of infectious hepatitis. Fourteen cases of hemolytic anemia were selected from the files of the Hematology Division, Department of Medicine, Washington University, St. Louis, Missouri; diagnoses had been established by appropriate laboratory data.

For analysis the cases were grouped into the following categories (Table 1): 1) diffuse hepatocellular disease, including toxic hepatitis, acute hepatic necrosis, infectious hepatitis, Laennec's cirrhosis of the liver, fatty infiltration of the liver; 2) hemolytic anemia, including congenital and acquired forms; 3) miscellaneous, including primary carcinoma of the liver, metastatic carcinoma of the liver, multiple abscesses of the liver, lymphosarcoma of the liver, acute and chronic cholecystitis without common duct obstruction; 4) extrahepatic obstruction of the common bile duct, including common duct stone, chronic pancreatitis and cancer of the biliary tract.

Only one random direct and total bilirubin was recorded from each patient. If several determinations were available the highest total bilirubin was selected. No attempt to establish the onset of disease was made. All of the routine pathological data were used with the approval of the Snodgras Laboratory, St. Louis City Hospital. The total and direct bilirubin determinations were done by the Snodgras Laboratory and the Barnes Hospital laboratory using the method of Evelyn and Malloy[24], and Thannhauser and Anderson[16] respectively.

TABLE 1.—CLASSIFICATION OF DATA

Diagnoses		*Number of Cases*
1. Diffuse Hepatocellular Disease		50
a. Toxic hepatitis	1	
b. Acute hepatic necrosis	1	
c. Infectious hepatitis	11	
d. Cirrhosis of the liver	29	
e. Fatty infiltration of liver	8	
2. Hemolytic Anemia		14
a. Congenital	2	
b. Acquired	12	
3. Miscellaneous		19
a. Primary carcinoma of liver	3	
b. Metastatic carcinoma of liver	6	
c. Multiple abscesses of liver	1	
d. Lymphosarcoma of liver	1	
e. Acute cholecystitis without common duct obstruction	2	
f. Chronic cholecystitis without common duct obstruction	6	
4. Extrahepatic Obstruction of the Common Bile Duct		32
a. Common duct stone	13	
b. Chronic pancreatitis	1	
c. Cancer of the bile tract	18	
Total		115

The groups were analyzed, comparing the total bilirubin and the ratio

$$\frac{\text{Direct bilirubin}}{\text{Total bilirubin}} \times 100 \text{ (Watson}^{33}\text{)}$$

by the analysis of variance "F" ratio method (Croxton and Cowden[8]) using an I.B.M. 650 computer by the Computer Center, Washington University, St. Louis, Missouri, under the direction of Professor Kenneth Barton. The hypothesis that there is no significant difference between the total serum bilirubin and the ratio

$$\frac{\text{Direct bilirubin}}{\text{Total bilirubin}} \times 100$$

values recorded in the above four groups was tested. The level of significance (P) of 0.001 was selected to minimize the type I errors. These values were interpreted from standard

TABLE 2.—STATISTICAL DATA
ANALYSIS OF VARIANCE "F" RATIO

TOTAL SERUM BILIRUBIN

Groups	*"F"*	*Degrees of Freedom*		*Probability*	*Significant*
1–4	19.43	DF1 = 1	DF2 = 80	Less than 0.001	Yes
1–2	2.214	DF1 = 1	DF2 = 62	More than 0.01	No
2–3	6.45	DF1 = 1	DF2 = 31	More than 0.01	No
2–4	13.347	DF1 = 1	DF2 = 44	Equals 0.001	*
2–1,3,4	3.775	DF1 = 1	DF2 = 113	Equals 0.05	No
Within Group 4 Diseases 1–3	13.98	DF1 = 1	DF2 = 30	Less than 0.001	Yes

RATIO DIRECT BILIRUBIN × 100
TOTAL BILIRUBIN

Groups	*"F"*	*Degrees of Freedom*		*Probability*	*Significant*
1–4	8.79	DF1 = 1	DF2 = 80	0.001–0.01	No
1–2	61.46	DF1 = 1	DF2 = 62	Less than 0.001	Yes
2–3	47.75	DF1 = 1	DF2 = 31	Less than 0.001	Yes
2–4	169.08	DF1 = 1	DF2 = 44	Less than 0.001	Yes
2–1,3,4	97.57	DF1 = 1	DF2 = 113	Less than 0.001	Yes

*More data needed to determine significance; probably not significant.

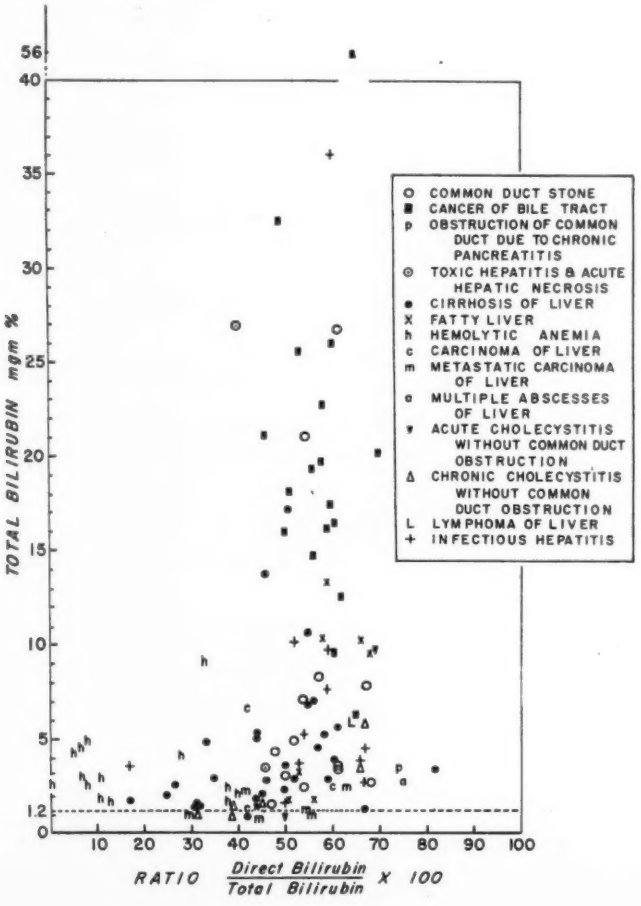
TOTAL BILIRUBIN mgm %
RATIO Direct Bilirubin / Total Bilirubin X 100
COMMON DUCT STONE
CANCER OF BILE TRACT
OBSTRUCTION OF COMMON DUCT DUE TO CHRONIC PANCREATITIS
TOXIC HEPATITIS & ACUTE HEPATIC NECROSIS
CIRRHOSIS OF LIVER
FATTY LIVER
HEMOLYTIC ANEMIA
CARCINOMA OF LIVER
METASTATIC CARCINOMA OF LIVER
MULTIPLE ABSCESSES OF LIVER
ACUTE CHOLECYSTITIS WITHOUT COMMON DUCT OBSTRUCTION
CHRONIC CHOLECYSTITIS WITHOUT COMMON DUCT OBSTRUCTION
LYMPHOMA OF LIVER
INFECTIOUS HEPATITIS

Fig. 1.

statistical tables (Croxton and Cowden[9]) (Table 2).

Results. From Table 2 and Fig. 1 it can be noted that the total serum bilirubin of the extrahepatic obstruction of the common bile duct is significantly higher than the diffuse hepatocellular disease group. However, any other comparison of the total serum bilirubin between these four groups is not significant. Within the obstructive group, the cases of cancer of the bile tract show significantly higher total serum bilirubin than those with common duct stones.

The ratio of direct over total serum bilirubin in the hemolytic anemia group is significantly different from the diffuse hepatocellular disease, miscellaneous and extrahepatic obstructive groups but the latter three cannot be differentiated.

In 8 cases the direct serum bilirubin was elevated above 0.2 mg. per 100 ml. but the total bilirubin was within normal limits (one primary carcinoma of the liver, three metastatic carcinoma of the liver, one acute cholecystitis and one chronic cholecystitis without common duct obstruction, and two cirrhosis of the liver).

Conclusion. Our data and analysis support the conclusions of others (Gray[11], Gray and Whidborne[12], Hoffbauer, Rames and Meinert[18], Klatskin and Drill[21], With[34], Zieve *et al.*[37], Zinninger[38]), that the determination of the direct serum bilirubin in conjunction with the total serum bilirubin does not materially aid in differentiating jaundice due to extrahepatic obstruction from that due to diffuse hepatocellular disease. Fractionation of serum bilirubin does appear to be of value in differentiating hemolytic anemia from these. Patients with obstruction of the common bile duct have serum bilirubin elevation more marked than values noted in other liver disease, with obstruction due to carcinoma generally causing more marked elevation than that due to common duct stone.

The routine use of the quantitative fractionation of serum bilirubin does not seem justified.

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SUMMARIO IN INTERLINGUA

Un Analyse del Application Clinic del Reaction de van den Bergh in Jalnessa

Nostre datos e nostre analyse supporta le conclusiones de alteres (Gray[11], Gray e Whidborne[12], Hoffbauer, Rames, e Meinert[18], Klatskin e Drill[21], With[34], Zieve *e al.*[37], e Zinninger[38]) que le determination del directe bilirubina seral in conjunction con le total bilirubina seral non contribue significativemente al differentiation inter jalnessa causate per un obstruction extrahepatic e jalnessa causate per diffuse morbo hepatocellular. Tamen, le fractionation del bilirubina seral pare esser de valor in distinguer anemia hemolytic ab le mentionate formas de jalnessa. Patientes con obstruction del commun via biliari ha elevationes del bilirubina seral de grados plus marcate que illos notate in altere morbos hepatic, e—a generalmente parlar—obstruction per carcinoma causa un plus marcate augmento que obstruction per calculo del commun via biliari.

Le uso routinari de fractionation quantitative del bilirubina seral non pare esser justificate.

STUDIES ON GROWTH HORMONE: V. EFFECT ON THE MINERAL AND NITROGEN BALANCES OF BURNED PATIENTS

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A prolonged catabolic response to a burn injury results in the loss of large amounts of body tissue. Delayed wound healing and an increased susceptibility of the burned patient to infection are problems that appear to be related to the magnitude and duration of the catabolic response. Practical means have been sought whereby this response could be minimized. If this can be accomplished, many of the problems common to the postburn course may be eliminated.

Pituitary growth hormone (GH) has been reported (Bartlett[1], Russell[9]) to promote growth and to bring about nitrogen, phosphorus, and calcium retention in experimental animals. These effects have not been consistently demonstrated in human subjects, and reports have appeared in which the effectiveness of bovine GH in the primate is questioned. However, an apparent anabolic effect has been reported to follow the administration of bovine GH to humans (Carballiera *et al.*[2], Prudden, Pearson and Soroff[5], Shorr *et al.*[10]), and it is a cardinal premise of the present study that effectiveness of this preparation is dependent upon a sufficiently high dosage of the bovine preparation.

Carballiera and associates[2] observed a lowering of the blood amino acid level and of urinary amino acid excretion following the intravenous administration of 70 to 225 mg. of GH in an amino acid solution. They felt that these changes, in the absence of a significant increase in plasma nonprotein nitrogen, were indicative of increased utilization of the administered amino acids. These data are compatible with studies of protein synthesis by the perfused rabbit liver with varying levels of amino acid substrate (Price and Prudden[4], Prudden, Young and Stirman[6]). The perfusion data indicated that GH is a much more efficient stimulator of protein synthesis than insulin when the alpha amino nitrogen concentration in the perfusate is high;

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whereas, at lower levels, GH was less effective than insulin (Price and Prudden[4]).

In a previous report, an analysis of the nitrogen balance data in burned patients (Prudden, Pearson and Soroff[5]) demonstrated that GH tended to increase nitrogen retention during the anabolic phase of convalescence providing the nitrogen intake was above 10 gm. per square meter of body surface. Lower levels of nitrogen intake concomitant with GH administration resulted in a decreased nitrogen retention. These findings suggest that an adequate level of amino acid nitrogen is necessary to elicit an anabolic effect from the administration of GH.

This report concerns the mineral balance data that were obtained in conjunction with the nitrogen study previously reported (Prudden, Pearson and Soroff[5]).

Patient Material. Patient No. 8* was a 59-year-old woman who sustained third-degree burns of 15% of the body surface with a total burn of 30%. The second-degree burns healed well and the patient was transferred to this hospital on the 16th postburn day. On postburn day 29, split-thickness grafts were applied to all the granulating areas. Final grafting procedures were performed on postburn days 72 and 138. Balance study began on postburn day 18, the late acute phase, and was continued through 21 consecutive periods consisting of one 7-day and twenty 5-day periods. During periods 4, 6, 8, 12, and 16, 150 mg. of GH† were administered intramuscularly each day. The patient had mild discomfort at the site of the injection of the growth hormone throughout the study and during the last GH period developed severe itching, nausea, and periorbital edema.

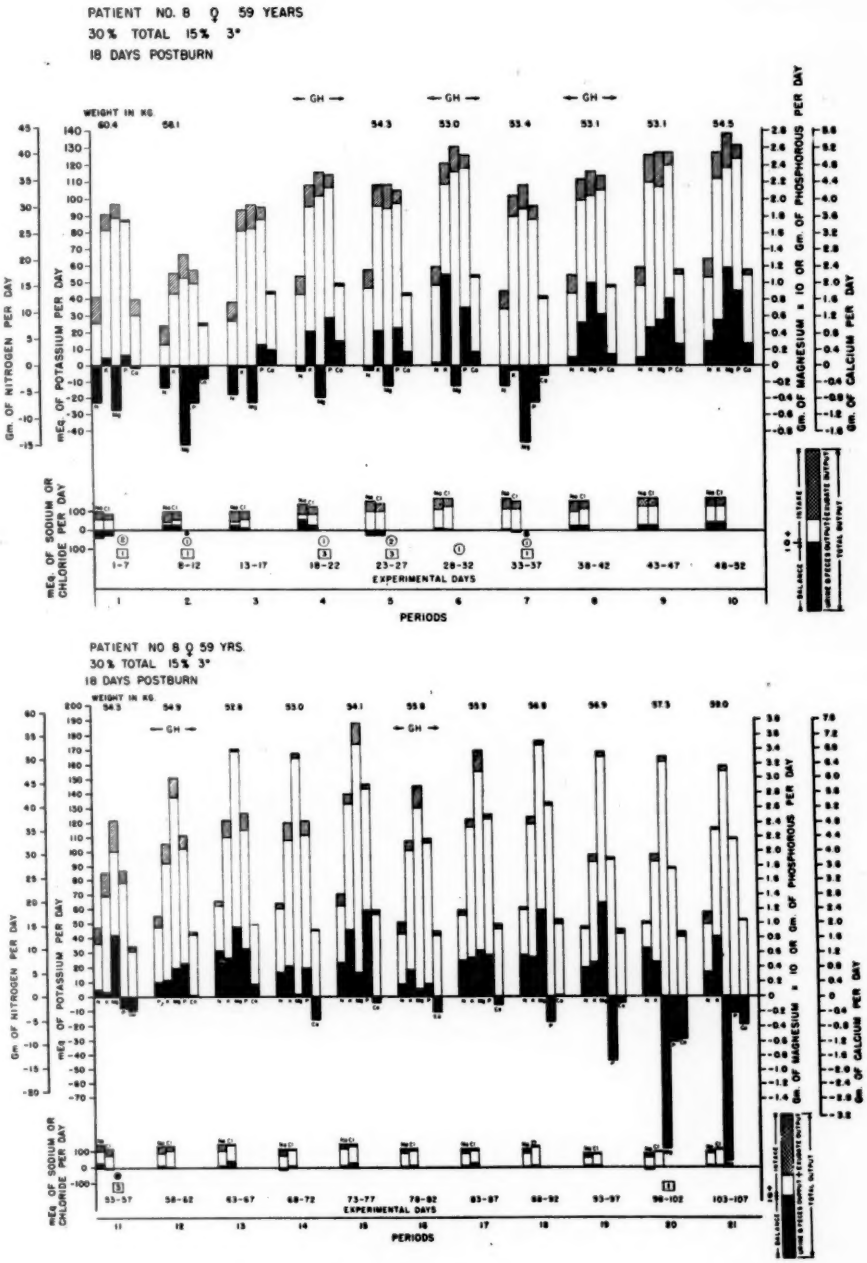
Patient No. 11 was a 22-year-old man who sustained third-degree burns of 35% of the body surface with a total burn of 55%. On postburn day 23 he was transferred to this hospital. Balance study began on postburn day 47, the early convalescent phase. All of the second-degree areas had healed at this time, but granulating areas covered 35% of the body surface. The study was continued through 12 consecutive 5-day periods. During periods 2, 4, 6, 8, and 10 the patient was given 200 mg. of GH intramuscularly each day.

Patient No. 10 was a 20-year-old man who sustained third-degree burns of 35% of the body surface with a total burn of 50%. On postburn day 45 skin grafts were applied to approximately 75% of the granulating areas. On postburn day 75, he was transferred to this hospital. Further grafting was performed on postburn days 82 and 102. The wounds were healed by postburn day 113. Balance study began on postburn day 124 during the late convalescent phase and was continued through 59 consecutive days with one 4-day and eleven 5-day periods. The patient's preburn weight was 80 kg., and at the beginning of the study his weight was 56 kg. During periods 4, 9 and 11, the patient was given 200 mg. of GH intramuscularly each day.

Patient No. 9 was a 20-year-old man who sustained third-degree burns of 40% of the body surface. On postburn days 28 and 38, the entire granulating area was covered and the graft take was estimated at 80 to 90%. He arrived at this hospital on postburn day 53. At this time the donor sites were purulent and had converted to full-thickness loss. The patient's oral intake was poor and tube feeding could not be accomplished satisfactorily because of recurrent vomiting. The nutritional state of the patient deteriorated and the wounds remained unhealed despite repeated grafting procedures. At intervals between postburn days 181 and 260, cadaver homografts were applied in an attempt to reduce temporarily the granulating area until sufficient autografts were available. By postburn day 281, only a few small granulating areas remained which gradually healed without further autografting and the patient was then progressively rehabilitated. Balance study began on postburn day 86 and continued initially for four 5-day periods. Following a 5-week interval, the metabolic study was resumed and continued for 32 consecutive 5-day periods. During periods 6, 9, 12, 17, 24, and 33,

*The patients' numbers refer to their assigned numbers in the metabolic unit rather than to their sequence in this particular investigation.

†Growth Hormone, Lot #R491025. This material was generously supplied by Dr. C. J. O'Donovan, Assistant Medical Director of The Armour Laboratories. The only significant contaminant was reported to be 0.32 ± 0.098 unit of thyrotropin per mg.



Figs. 1*A* and 1*B*.—Metabolic relationship chart of Patient 8. The study began on postburn day 18.

The symbols under some of the sodium and chloride balances indicate which of the following procedures took place during that period:

- ● = skin grafting
- ○ = dressing changes under general anesthesia
- □ = units of blood transfusion

the patient was given 200 mg. of GH intramuscularly each day.

Methods. BALANCE TECHNIQUES. The usual balance techniques were carried out (Reifenstein, Albright and Wells[7]). An *ad libitum* intake was offered. The composition of the foods offered and rejected was obtained by chemical analysis. Urine, feces, exudate, and emesis were collected and analyzed. Nitrogen, sodium, and potassium balances were obtained for all of the patients. In addition, calcium, phosphorus, magnesium, and chloride balances were determined for Patients Nos. 8 and 9.

METHOD OF CHARTING. An example of the balance data is shown in Fig. 1. Intake is plotted from the zero line up, output from the intake line down. Black areas below the zero line show negative balances, and those above the zero line positive balances. The cross-hatched area in each column is the exudate contribution to the output. In all figures the relative scales used in plotting the nitrogen and potassium balances are in the approximate ratio of these elements in protoplasm (Reiss, Pearson and Artz[8]). In Fig. 1, magnesium and phosphorus are charted in their respective protoplasmic ratios to nitrogen, and calcium and phosphorus are plotted in a ratio that approximates that of bone. The periods during which the GH was given are indicated in the figure.

Results. BALANCE DATA. The studies of Patients Nos. 8, 11, and 10 were begun at times representing the late acute phase and the early convalescent phase and the late convalescent phase, respectively, of the postburn course. Patient No. 9 did not fit into any of these categories since his postburn course was unusually protracted. At the time the study began, on postburn day 86, the patient was extremely debilitated.

The balance data of Patient No. 8 was characteristic of the metabolic response to a moderate burn (Fig. 1). The catabolic phase, as defined by the duration of negative nitrogen balance, was approximately 45 days. During the anabolic phase the retention of all of the elements fluctuated with the calcium, phosphorus, and magnesium balances becoming negative during Periods 19, 20, and 21.

The study of Patient No. 11 was begun during early convalescence while his wounds were still granulating. The patient was in negative nitrogen balance during the first two study periods and in positive nitrogen balance thereafter. The sodium balance was slightly negative during periods 3 and 7, but the potassium balance was positive throughout the entire study.

Patient No. 10 was entirely healed when the study was begun late in his convalescence from a severe burn. The patient showed a positive nitrogen balance with retention of potassium and sodium throughout the study.

Patient No. 9 had a positive nitrogen balance on a moderate intake during the first four study periods. When the studies were resumed after a 5-week interval, the nitrogen balance was negative and remained so except for isolated instances until Period 19. During the early part of the study the potassium balance was frequently positive in the presence of a negative nitrogen balance. In general, a positive nitrogen balance preceded positive magnesium, calcium, and phosphorus balances. The sodium and chloride balances fluctuated during the first half of the study.

WEIGHT CHANGES. The average weight during each period is recorded on the metabolic charts. In Patient No. 8 the weight remained fairly constant throughout the first half of the study and then gradually increased until the end of the study when it approached the preburn weight of 60 kg. Although no consistent correlation relates weight gain to GH administration, the largest weight gain for this patient occurred during the last period in which GH was administered, and in spite of a decreased intake. There was no apparent relationship between weight gain

or loss and GH administration for any of the other patients in this study.

URINARY 17-KETOSTEROIDS AND 11-OXYCORTICOIDS. The urinary 11-oxycorticoids were elevated above normal in the beginning of the study in Patients Nos. 8 and 10, and throughout most of the study in Patient No. 11. The urinary 17-ketosteroids were below or within the normal limits for these 3 patients during the period of study. There was no increase in 17-ketosteroid excretion during any of the GH periods, thereby providing clinical evidence against the occurrence of significant ACTH contamination of the GH preparation used.

COMMENTS. *Theoretical Balances.* There were many periods during the study of each patient when the potassium balance was more positive than would be expected on the basis of the nitrogen balance. This is shown clearly in Fig. 1. If potassium and nitrogen loss or storage had occurred in the protoplasmic ratio, the black areas indicating the balances would be of the same height because of the method of charting. The differences in rates of storage were especially apparent in the balance charts of Patients Nos. 8 and 10 (Fig. 1). The phosphorus and nitrogen balances were also charted in the protoplasmic ratio. In the figure the phosphorus balance can be visually corrected for loss or storage with calcium by algebraically subtracting the calcium balance from the phosphorus balance. Figure 1 shows a relative phosphorus retention during many periods of the study. This was also true of Patient No. 9.

Similar discrepancies in the relative nitrogen and mineral loss and storage in the burned patient have been reported by Reiss, Pearson and Artz[8]. The pattern of relative retention of potassium and phosphorus did not appear to be altered during the periods of GH administration (Fig. 1).

Analysis of Data. The technique of covariance analysis was used to examine the difference between the balances of the non-GH and GH periods since a dependence of balances on intake has been previously reported (Prudden, Pearson and Soroff[5]), covariant analysis permitted an elimination of the effect on the balances of the varying *ad libitum* intake. Thus it was possible to separate the effect of GH on the mineral balances from the effect of different mean levels of intake during the GH and non-GH periods. This technique also permitted the calculation of mean mineral balances for the several patients, on the hypothetical basis of a common level of intake. An example of the calculations is presented in Tables 1 and 2 based on the potassium data of Patient No. 8. The intakes and balances for each mineral element were analyzed for the individual patient by this method. It should be noted that calcium, phosphorus, magnesium, and chloride balances were available for only Patients Nos. 8 and 9. Since the GH periods of Patients Nos. 8, 9, and 10 were widely separated in time, only the non-GH periods immediately preceding and succeeding the GH periods were utilized in the analysis. All of the periods of Patient No. 11, except the last, were used in the calculations since the study consisted of alternate non-GH and GH periods but terminated with two non-GH periods.

POTASSIUM. The relationship of the potassium balance to the intake in Patient No. 8 is illustrated in Fig. 2. It is apparent that the regressions of the potassium balance *versus* intake have different slopes for the GH and non-GH periods and in this instance, the difference was significant ($0.5>P>0.01$). A similar, but non-significant

TABLE 1.—SUMMARY OF POTASSIUM INTAKE (X) AND BALANCE (Y) PER SQUARE METER OF NON-GH* AND GH PERIODS OF PATIENT NO. 8

Periods			
Non-GH		*GH*	
X mEq./m²	*Y* mEq./m²	*X* mEq./m²	*Y* mEq./m²
54	−1	63	+12
63	+12	70	+32
59	−2	64	+15
73	+13	59	+6
48	+2	60	+10
68	+15		
78	+26		
68	+14		

*Non-GH periods immediately preceding and succeeding the GH periods.

TABLE 2.—COVARIANT ANALYSIS OF POTASSIUM DATA OF NON-GH* AND GH PERIODS OF PATIENT NO. 8

		Sums of Squares and Products				*Errors of Estimate*		
Source of Variation	*Degrees of Freedom*	x^2	xy	y^2	*Regression Coeff.*	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*
Non-GH periods	7	691	583	639	0.8437	6	147	2.4
GH Periods	4	75	170	404	2.2667	3	19	6.3
Sum						9	166	18.4
Between Reg. Coeff.						1	137	137.0
Average within Periods	11	766	753	1043	0.9830	10	303	30.3
Between adjusted Means						1	104	104.0
Total	12	767	742	1124	0.9674	11	407	

*Non-GH periods immediately preceding and succeeding the GH periods.

F, Regression $\frac{137.0}{18.4} = 7.4456$ $0.05 > P > 0.01$

F, Balances $\frac{114.0}{30.3} = 3.4323$ $0.2 > P > 0.1$

TABLE 3.—SUMMARY OF REGRESSION COEFFICIENTS OF NON-GH* AND GH PERIODS

		Mineral Element					
Patient	*Period*	*K*	*Na*	*Cl*	*P*	*Mg*	*Ca*
.8	Non-GH	0.8437	−0.0326	0.2062	1.1767	0.6654	0.4894
	GH	2.2667	0.0073	−0.2628	1.0406	−0.1021	1.6078
.9	Non-GH	−0.0408	0.5548	−0.3790	0.7045	−0.6749	−0.3050
	GH	0.3001	−0.9682	−0.4952	0.7413	2.6979	0.3776
.10	Non-GH	0.0536	0.1070				
	GH	0.4141	0.7032				
.11	Non-GH	0.6926	2.1808				
	GH	0.2724	0.5308				

*Non-GH periods imme preceding and succeeding the GH periods.

difference was observed for Patients Nos. 9 and 10 (Table 3).

The intersection of the non-GH and GH regression lines is termed the "critical level" of intake (Reifenstein, Albright and Wells[7]). A more positive potassium balance occurs with GH administration than without it when the intake is above this critical level, but with an intake below this level the reverse is true. For all of the patients this critical level was found to approximate the average *ad libitum* intake. This suggests that the level of intake consumed voluntarily during the study was not sufficient to elicit a maximal therapeutic effect from the administration of GH on the mineral balances, and forced feeding would be desirable.

Of all the minerals studied, the most pronounced and consistent effect of GH was seen on the potassium balances. Effects of a less reproducible nature were found in the other mineral data.

SODIUM CHLORIDE. In Patient No. 9 the trend in the sodium balances and chloride balances were parallel. During the GH periods of this patient the regression of both sodium and chloride on intake showed a progressively negative influence of the intake on the balance, that is, less sodium and chloride retention was seen during the GH periods. A similar effect was seen in the chloride data of Patient No. 8 and in the sodium data of Patient No. 11.

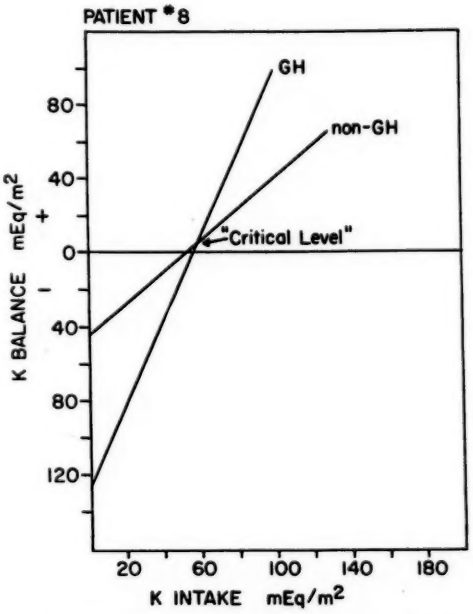


Fig. 2.—The relationship between the potassium balance and the potassium intake for all data with and without growth hormone.

CALCIUM, MAGNESIUM AND PHOSPHORUS. The regressions of the calcium balances of both Patients Nos. 8 and 9 were steeper during the GH periods and in Patient No. 9 this trend was significant when compared with the regression of the non-GH periods. As in the case of potassium there was a

"critical level" of calcium intake, similar to the average *ad libitum* intake, above which GH would seem to enhance calcium storage.

Patient No. 9 also had a significantly steeper slope in the regression of the magnesium balances during the GH periods, whereas, the slope of the magnesium balances of Patient No. 8 became negative during the GH periods.

The regression lines of the phosphorus balances were similar for the non-GH and the GH periods for both patients. There was an increase in the phosphorus balance with an increase in the intake, but this increase was not different when GH was given.

The results of this study made of calcium, phosphorus, and magnesium are suggestive, but because of the small number of patients involved (2), this study is only at its initial stage.

NITROGEN AND POTASSIUM BALANCES COMBINED FOR ALL PATIENTS. The nitrogen and potassium data of the GH periods of the patients were combined in an effort to compare the response to GH of patients who represented different phases of the postburn course. For validity of comparison, intake and balance were converted to intake and balance per square meter. The balances were adjusted to a common intake by an extension of the covariant analysis. Significant differences were observed as indicated below.

Patient No. 10, studied in the late convalescent phase, had the most positive nitrogen balance during GH administration and Patient No. 9, the debilitated patient, a negative balance. This observation is compatible with the previous report that GH administration results in an increased nitrogen storage during the anabolic phase (Prudden, Pearson and Soroff[5]). When the potassium balances were adjusted to a common intake, Patients Nos. 8 and 11, who were studied earliest in the postburn course, had the most positive balances. This is in agreement with the observation that potassium repletion occurs more rapidly than nitrogen repletion (Reiss, Pearson and Artz[8]).

While the differences observed can be attributed to the different postburn phases studied, the necessarily small numbers of individuals studied make final statements difficult.

Further studies are in progress on burned patients of comparable age, sex, and extent of burn in an attempt to separate the effects of changing intake and the stage of convalescence from the effects of GH.

Animal studies now in progress in this laboratory (Gump, Schwartz and Prudden[3]) show essentially similar effects upon the metabolism of burned rats receiving bovine GH. This is of particular interest because of the unquestioned effectiveness of the bovine growth hormone preparation in this species.

Conclusions. 1. The administration of GH to burned humans results in an overall significant effect upon the potassium balance. In the patients studied, a "critical level" of intake was noted, above which the hormone increased the retention of potassium over the control periods, and below which the reverse was true. This "critical level" approximated the mean *ad libitum* intake over the entire period of the study.

2. The effect of GH administration upon calcium balance was similar to that observed for potassium, although documented in a smaller number of patients.

3. A parallel trend was exhibited by sodium and chloride, less retention of these minerals being observed during GH administration.

4. No discernible effect of GH upon the magnesium and phosphorus bal-

ance was observed for the two patients for which these balances were studied.

Summary. The effect of intramuscularly administered GH on potassium, sodium, and nitrogen balances was studied in 4 burned patients. In addition, calcium, phosphorus, magnesium, and chloride balances were measured in 2 of the patients. The studies of 3 of the patients were begun at different postburn times representing the late acute phase and the early and late convalescent phases. The fourth patient was debilitated and could not be classified in this manner.

Covariant analysis was used to compare balances of the non-GH and GH periods of each mineral element for each patient taking into account the different levels of *ad libitum* intake to each patient. This analysis indicated that an increase in intake of potassium and calcium was associated with a greater increase in the balances during the GH than during the non-GH periods if the intake surpassed a "critical level." The "critical level" was found to approximate that of the average *ad libitum* intake.

A trend to decreased retention of sodium and chloride during GH administration was observed; but no consistent trends were discernible in the phosphorus and magnesium data.

When the mean nitrogen and potassium balances of the GH periods of the 4 patients were adjusted to a common intake, the patient studied in late convalescence had the most positive nitrogen balance and the 2 patients studied earliest in the postburn course had the most positive potassium balances. This is in accord with our previous observations on the effect of GH during various stages of convalescence.

ACKNOWLEDGMENT: The authors wish to express their gratitude to Lieutenant Colonel Curtis P. Artz, MC, for his help and advice. Corporals Harlan Jorgensen, Alfred P. Howard, and Monroe Nair contributed valuable technical assistance. Captains Reba Holland, Helen Donnelly, Margaret Mahar, and Edith Bonnet, and Lieutenant Alice Edelen were responsible for the nursing care and for carrying out the metabolic routines meticulously.

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SUMMARIO IN INTERLINGUA

Studios Relative a Hormon de Crescentia V: Effecto Super le Balancia de Minerales e de Nitrogeno in Patientes con Lesiones per Arditura

Le effecto exercite per administrationes intramuscular de hormon de crescentia super le balancia de kalium, de natrium, e de nitrogeno esseva studiate in 4 patientes ardite. In plus, le balancia de calcium, de phosphoro, de magnesium, e de chloruro esseva mesurate in 2 del patientes. In 3 del casos, le studios esseva comenciate a differente periodos con respecto al tempore del vulneration, *i.e.* in le tardive phase acute, le precoce phase de convalescentia, e le tardive phase de convalescentia. Le quarte del patientes esseva debilitate e non poteva esser classificate in le mesme maniera.

Le methodo del analyse covariantial esseva usate in comparar le balancias inter le periodos con e sin hormon de crescentia pro le varie elementos mineral in le caso de omne patiente individual, con consideration del differente nivellos del non-restringite ingestion. Iste analyse indica que un augmento del ingestion de kalium e de calcium esseva associate con un plus grande augmento del balancias durante le periodos con hormon de crescentia que durante le periodos sin hormon de crescentia si le ingestion excedeva un certe "nivello critic." Esseva trovate que le "nivello critic" esseva approximativemente le media del non-restringite ingestiones.

Esseva notate un tendentia del retention de natrium e chloruro de reducer se durante le administration de hormon de crescentia sed nulle persistente tendentias esseva manifeste in le datos pro phosphoro e magnesium.

Quando le balancias medie de nitrogeno e de kalium durante le periodos con hormon de crescentia in le caso del 4 patientes esseva adjustate al mesme nivello de ingestion, le patiente studiate durante le periodo tardive de convalescentia habeva le plus positive balancia de nitrogeno, e le 2 patientes studiate plus tosto in lor curso post-arditural habeva le plus positive balancias de kalium. Isto es de accordo con nostre previe observationes relative al effecto de hormon de crescentia in varie stadios de convalescentia.

SUMMARIO IN INTERLINGUA

(See page 61 for original article)

Le Test Intravenose a Rubio Congo: Un Description de Certe Non-Recognoscite Fallacias con Proponimentos de Melioration

1. Le accuratia del test intravenose a rubio congo esseva investigate. Es presentate rationes pro le inaccuratias revelate.

2. Es proponite modificationes pro compensar iste deficientias. Es sublineate le importantia de preparar solutiones standard con sero e etiam le disavantages del uso de acetona como agente in le tractamento del sero.

3. Esseva executate un studio del test in un serie de patientes con arthritis e altere disordines. Le resultatos monstra que in certe casos de arthritis rheumatoide il occurre un augmentate absorption histic del colorante. Si iste phenomeno es plus frequente in arthritis rheumatoide que in altere morbos pare esser dubitabile.

STUDIES ON GROWTH HORMONE: VI DEPENDENCE OF ANABOLISM ON THE LEVEL OF INTAKE*

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and

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A number of clinical evaluations of growth hormone have been conducted in order to evaluate its effect, if any, on human nitrogen balance (Kinsell[4], Moore[5]). These studies were carried out with bovine growth hormone, and the results obtained, together with studies on pituitary dwarfism utilizing the same preparation (Shorr *et al.*[11]), have led to the widespread conclusion that growth hormone of bovine origin is inactive in man. Recently, attention has been focused on the much greater activity in humans of monkey or human growth hormone (Beck *et al.*[2]). However, the problem of an adequate supply of the primate material remains unsolved.

In 1956, Prudden, Soroff and Pearson[9] evaluated the effects of high dosage of bovine growth hormone† by means of a detailed study of the nitrogen balances of 4 severely burned patients. The dosage used was 50 mg. of GH in isotonic saline injected intramuscularly every 6 hours throughout each 5-day metabolic period studied in each of the patients. It was concluded that at this dosage level bovine growth hormone had exerted a reproducible metabolic effect on these human subjects, specifically that GH had brought about an increase in the retention of nitrogen during anabolic states, but had deepened existing catabolic states. It was observed that the anabolic effect was always associated with a relatively high food intake (more than 10 to 12 gm. of nitrogen per square meter of body surface); and it was concluded that large intakes were necessary in order to achieve increased positive nitrogen balance during GH administration.

The apparent necessity for an optimally high intake to produce the anabolic effect of GH is especially interesting in view of the current studies by Allison[1] on the positive influence of caloric intake upon the rate of replenishment of depleted protein reserves. The deepening of catabolism which GH shows under certain circumstances also seems analogous to the catabolic states which may be brought about by disproportionate excess of certain amino acids ("amino acid imbalance") (Harper[3]). In brief, this would indicate that the effect of GH must be related to the nutritional status of the patient in question. It is not an absolute anabolic agent. Such agents do not seem to exist.

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†Hereafter, "GH" will indicate only this growth hormone preparation.

Because of the conclusion that GH of bovine origin had been active in the human in all instances when the "50 mg. every 6 hour" level of dosage had been employed, it appeared important to re-study the results of bovine GH administration with experimental animals in a "clinical" status similar to that which had characterized the burn patients. The necessity of a high food intake to the anabolic-action of growth hormone could be tested by varying the food intake at an appropriate point in the post-burn course. Irrespective of the relationship of the experimental study to the clinical observations, the former should provide new information on the activity of growth hormone under previously uninvestigated conditions in an animal recognized as GH-sensitive.

Methods. Seventy-six male Sherman strain rats were grouped in a random manner, given identifying markings, and distributed two to a cage. Half the cages were used for the control animals, and the other half served as tests. The growth of the animals (whose average age, calculated from initial weight by the tables given by Spector[12], approximated 10.4 weeks) was followed over an 18-day control period during which weight and food and water intakes were measured 3 times a week. At the end of this time, all the rats were anesthetized with Nembutal (40 mg. per kg.), shaved, and their backs burned over a 7 × 4 cm. area. The procedure was facilitated by placing the anesthetized rat on its back in an asbestos lined cylindrical container with a 7 × 4 cm. aperture which was then lowered into the water bath. In order to obtain uniform 3rd degree burns, a constant temperature water bath at 70° C. was employed, and the rats were immersed for 40 seconds. The 7 × 4 cm. burn caused no appreciable mortality in either the test or control groups.

Beginning with the day of burning, and daily thereafter until the experiment was terminated, 4 mg. of GH* in 2 ml. of saline was administered intramuscularly to each of the rats in the experimental groups, while the control received an identical intramuscular injection of saline. The animals had unlimited access to Rockland animal pellets and tap water. Daily weights and daily food and water intakes were recorded.

Two weeks following the burn, food was completely removed from all cages and the rats were maintained on *ad libitum* water and salt. GH and saline injections were continued as before, and the daily weights and water intakes were recorded for the next 10 days.

Results. The average weights of the control and test groups have been plotted against time in Fig. 1. It can be seen that the two groups diverge sharply following the post-burn GH administration to the test group. Three cages from the control series were discarded because of deaths following burning. There were no deaths during this time in the GH group.

The weight variations over the first 9 postburn days were averaged in order to arrive at an average daily weight change, or ΔW, for each animal. Since the daily food intake figures were on a per cage basis, it was necessary to average the ΔW figures for the 2 rats in each cage in order to compare weight change with food intake. The results are shown in Figs. 2 and 3. One animal in the GH group that failed to gain weight and which was more than two standard deviations from the line shown in Fig. 1, was discarded ("Chauvenet criterion"). Covariant analysis of these data was undertaken in order to examine the precise relationship between food intake and weight change (see Table 1).

The analysis indicates that the slope of the linear relationship (Figs. 2 and 3) between ΔW and food intake was significantly steeper for the GH treated group of animals than for that of the controls ($.05 > p > .01$). The exact equations of the control and GH lines permitted a determination of the ΔW intercept at zero food intake for each

*The bovine growth hormone employed in this study was a gift of the Endocrine Study Section, Division of Research Grants, National Institutes of Health.

group. The intercepts were —3.31 gm. and —5.77 gm. for the control and GH groups, respectively. These intercepts represent predictions (extrapolations) of weight loss for each group on zero food intake; they predict a greater weight loss for the growth hormone treated group under conditions of zero food intake. The two lines were found to intersect one another when food intake and ΔW were 8.8 gm. and —1.88 gm., respectively. The point of intersection ("the critical point") represents the one situation where food intake and balances are identical for control and GH treated groups. With food intakes below this point, the controls consistently have greater weight increases than the GH-treated animals. With food intakes above this critical

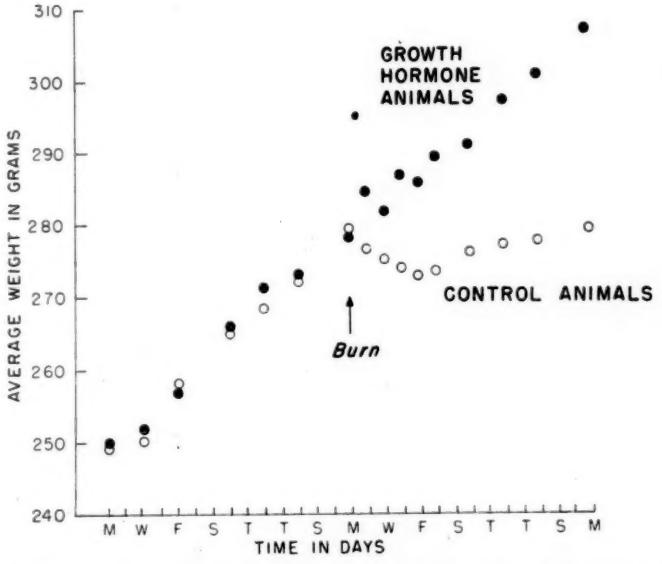

Fig. 1.—A graphical comparison between the mean weights of the two groups over time, both before and after burning and concomitant growth hormone administration.

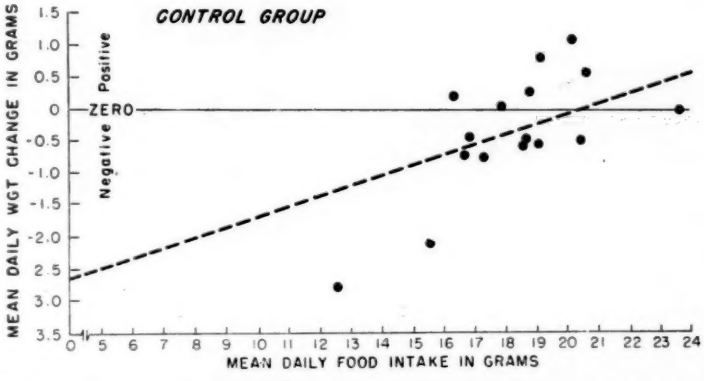

Fig. 2.—The fitted line representing the relationship between the mean daily weight change and mean daily food intake in the *control group*.

point, the GH-treated animals consistently have greater weight increases than the controls. This relationship agrees with clinical nitrogen and potassium balance data reported previously (Pearson *et al.*[7], Prudden, Pearson and Soroff[9]).

Added emphasis is given to the above findings when it is pointed out that there was no significant difference in food intake, *per se*, between the two groups of animals; indeed, the average daily intakes for each group were within 0.3 gm. (A similar analysis showed no significant differences in water intake and water retention between the two groups.)

The third and final phase of the experiment was initiated 2 weeks after the burn, at which time the food was removed from the cages. The majority of the animals in both groups were

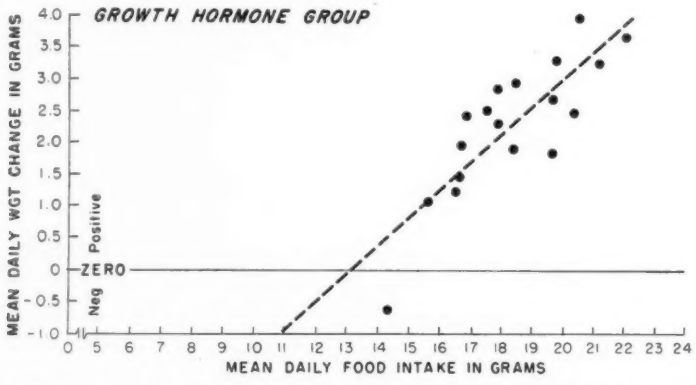

Fig. 3.—The fitted line representing the relationship between the mean daily weight change and mean daily food intake in the *growth hormone group*.

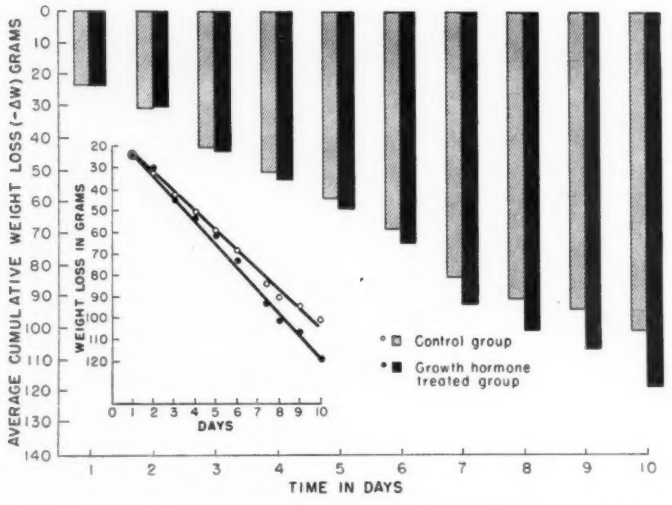

Fig. 4.—A line and bar graph representation of the cumulative weight loss in both experimental groups with time.

dead at the end of the 10-day period which followed, but a chi square test failed to demonstrate a significant difference between mortality of the control and GH animals. Figs. 4*A* and *B* are plots of the *average cumulative weight loss* for the two groups of animals *vs. time*. The slope for the GH treated group is steeper than that for the control group, and this difference between slopes is highly significant ($p<.001$) (Fig. 4*A*). Hence there is a more rapid weight loss on zero food intake for the GH-treated animals than for the controls. When the two groups were compared on a day to day basis it was found that the cumulative differences became significant ($p<.001$) on the 7th day, and remained so thereafter (Fig. 4*B*).

These experimental observations confirm the predicted result obtained by extrapolation of the weight-intake data to a zero intake (see above). The possibility that the greater initial weights of the GH group of rats were the cause for the greater cumulative weight loss was ruled out by a covariant analysis which took account of initial weight compared to the subsequent average weight loss.

Discussion. These results demonstrate the capacity of bovine GH to alter the post-traumatic metabolism of the rat. The postburn weight loss seen in the control group was eliminated by treatment with GH. Since both groups had essentially identical food intakes, one must assume that the GH rats must have *utilized* their food in a different fashion from the controls in order to explain the markedly different weight curves.

The GH and control regression lines calculated from the average daily food intake and ΔW data intersect at a point where food intake is 8.8 gm. per day. This is termed the "critical point." When daily food intake is greater than this figure, as it was in the first part of the experiment, the GH treated animals gain more weight than the controls. When food intake falls below this point, one would expect GH administration to cause an increased weight loss compared to control animals. This hypothesis was tested by reducing the food intake to zero, and the GH treated animals did indeed lose weight at a faster rate. This effect was not evident until the third day, and not significant until the seventh day, after withdrawal of food. The critical point phenomenon coincides with observations made in an earlier clinical study (Pearson *et al.*[7]).

The results reported above demonstrate that, in the rat, an animal known to be sensitive to the action of bovine growth hormone, the administration of the hormone will cause an *increased* loss of weight (and presumably of nitrogen) in the absence of adequate food intake. This was a principal observation in the clinical burn studies. It is also lent support by recent observations on the influence of caloric intake and amino acid imbalances upon nitrogen metabolism.

The similarity of the human and rat findings raises more insistently the question of whether the positive effects which have been demonstrated in humans depend, first, upon the higher doses of the bovine hormone employed, and second, on the conditions of employment (that is, level of food intake). It seems safe to conclude from the current study that GH requires a higher than normal turnover of metabolites in order to achieve a grossly anabolic state after injury. This is a facet to its activity which is also consistent with liver perfusion studies employing growth hormone (Price and Prudden[8], Prudden, Young and Stirman[10]).

Summary. 1) Bovine growth hormone in high dosage exerts a markedly anabolic effect on the post-burn metabolism of the rat, eliminating the loss of weight seen in the control group of animals. 2) This anabolic effect of growth hormone is dependent upon the level of caloric intake. 3) Administration of the hormone to rats with zero food intake resulted in a greater loss of weight than was shown by control animals under similar circumstances. 4) The similarity of this result to clinical experience in the use of high doses of bovine growth hormone in burned humans is noted.

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SUMMARIO IN INTERLINGUA

Studios Relative a Hormon de Crescentia VI: Dependentia del Anabolismo del Nivello de Ingestion

1. Bovin hormon de crescentia in alte doses exerce un effecto marcatemente anabolic super le metabolismo de rattos lesionate per ardituras e elimina le perdita de peso que es vidite in le animales de controlo.

2. Iste effecto anabolic de hormon de crescntia depende del nivello de ingestion caloric.

3. Le administration del hormon a rattos con un ingestion caloric amontante a zero resultava in un plus grande perdita de peso que illo notate in animales de controlo sub le mesme conditiones.

4. Es notate le similitude inter iste resultato e le experientia clinic in le uso de alte doses de bovin hormon de crescentia in patientes human qui ha suffrite lesiones per arditura.

SUSCEPTIBILITY OF HEMOPHILUS INFLUENZAE TO 21 ANTIBIOTICS *IN VITRO**

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There is a continuous and growing interest in the potential decreases in susceptibility of various pathogenic organisms to the antibiotics that are in common use. Much of this interest has been due to the increasing proportion of strains of *Staphylococcus aureus* isolated from both infected sources and carriers and found resistant to some of the most widely used antibiotics, namely, penicillin, streptomycin, and the tetracyclines, and, more recently, also to erythromycin and novobiocin. The possibility must always be considered that exposure of large numbers of the population to these antibiotics may result in the acquisition of resistance by the bacteria or in the gradual elimination of the more susceptible strains, permitting the more resistant ones to spread (Finland[2]). It is, therefore, important to review the status of some of the common varieties of potential pathogenic bacteria from time to time in order to be able to anticipate possible difficulties in antibiotic therapy.

Hemophilus influenzae is a useful organism for such surveillance. Strains of this organism have all been moderately or highly susceptible to all of the widely used antibiotics except bacitracin. They are frequently found in the nasopharynx of infants and children and in the respiratory tract of adults without evident infections but may give rise to serious respiratory infections and meningitis in infants and to acute respiratory tract infections or exacerbations of chronic bronchopulmonary infections in older age groups.

Studies dealing specifically with the susceptibility of recently isolated strains of *H. influenzae* to the antibiotics available at the time were reported from this laboratory on 2 previous occasions (Finland and Wilcox[3], Love and Finland[7]). Significant alterations were not found in the sensitivity of strains isolated 5 years apart to antibiotics with which they were tested on both occasions. The present study includes tests with the same agents and also with a number of newly in-

*Aided, in part, by a grant (E–23) from the National Institutes of Health.

troduced antibiotics some of them related to the earlier ones.

Materials and Methods. STRAINS. The organisms tested were isolated from materials submitted to the Bacteriological Laboratory of the Boston City Hospital in January and February, 1959, and were identified by standard methods.* Most of them were from the respiratory tract (sputum, tracheal aspirates, lungs, and nasal or pharyngeal swabs) of patients with bronchopulmonary infections, but 3 were isolated from blood, 2 from cerebrospinal fluid and 1 each from exudates of an infected ear, eye and wound. Only the strains from the blood and cerebrospinal fluid were specifically identified as type B.

ANTIBIOTICS. The essential information about the antibiotics used is shown in Table 1. Standard solutions of the antibiotics were prepared in a concentration of 8 mg. per ml., distributed into screw cap tubes and kept frozen at −20° C. One tube of each antibiotic to be used was thawed for each day's tests and then discarded; fresh standard solutions were prepared each month. Carbomycin† and chlortetracycline, however, were freshly prepared on the day when they were used.

ASSAYS. For the inocula, the organisms were grown in heated horse blood ("chocolate") broth, pH 7.2, for 24 hours in a "candle jar" at 37° C. A 2 mm. loopful of undiluted culture was streaked on segments of plates of chocolate agar, pH 7.2± into which 2-fold dilutions of the antibiotics had been incorporated. The plates were incubated at 37° C. for 48 hours in a sealed incubator in which candles were allowed to burn to extinction. The endpoint was taken as the highest concentration of antibiotic on which growth was markedly or completely inhibited. The endpoints were quite sharp except with a few strains which showed marked inhibition over several dilutions of penicillin. A comparative study of 6 such strains tested in parallel with serial 2-fold dilutions of the antibiotic in a yeast extract-hematin, blood broth, using different sizes of inocula indicated that the endpoints as read routinely in this study corresponded, within a single 2-fold dilution, to the bactericidal endpoint in the liquid medium (no growth on subculture to chocolate agar), after growth for 24 or 48 hours from an inoculum of an equal volume of a 10^{-3} dilution of the seed culture. The standard laboratory strains Streptococcus 98, Staphylococcus 209P and *Klebsiella pneumoniae* were included in each day's tests for control of activity of the different antibiotics; endpoints with these organisms remained the same within a single 2-fold dilution throughout.

Results. The results of all the assays are summarized in Fig. 1 and in Table 2. As in the previous studies, the strains appeared to be susceptible, for the most part, to a relatively narrow range of concentrations, which did not differ markedly from one antibiotic to another. However, there were exceptions, namely bryamycin and some of the antibiotics of the erythromycin group—carbomycin, oleandomycin, spiramycin, and leucomycin—were much less active; 25 μg. per ml. or more of each of these antibiotics were required to inhibit the strains in nearly all instances. Streptogramin and MK 111, on the other hand, were about as active as erythromycin, but about one-fourth of the strains required slightly higher concentration of the latter. Also, penicillin, on a weight basis, was still the most active of the antibiotics tested, more than one-half of the strains being markedly or completely inhibited by 0.4 μg. or less per ml. of this antibiotic and very few of the strains were equally suceptible to any of the other antibiotics except novobiocin.

Among the other groups of related antibiotics, colimycin (Di Nola and Salassa[1]), a new polypeptide agent of the polymyxin group, appeared to be appreciably less active than polymyxin B. The 4 tetracycline analogues were quantitatively very similar in activity, demethylchlortetracycline being slightly more active and chlortetracycline apparently slightly less active than the other two. In the case of chlortetracy-

*We are indebted to Miss Marion E. Lamb and Miss A. Kathleen Daly for isolating the strains and making them available for this study.

†Anonymous. Antibiot. & Chemoth., 3, 865, 1953.

TABLE 1.—ANTIBIOTICS USED IN THIS STUDY

Antibiotic	*Lot*	*Supplier*	*Form Supplied*	*Form Calculated*	*Remarks*
Penicillin	K2644	Bristol	potassium	base	
Chloramphenicol	201320B	Parke, Davis	base	base	
Polymyxin B	678K	Burroughs Wellcome	sulfate	base	10,000 units per mg.
Colimycin	CD7710	Warner-Chilcott	sulfate	sulfate	18,000 units per mg.
Tetracycline	7–8980	Lederle	hydrochloride	hydrochloride	
Oxytetracycline	85230–5100B	Pfizer	"	"	
Chlortetracycline	7–8979	Lederle	"	"	
Demethylchlortetracycline	7–6693	"	"	"	
Streptomycin	03B0203	Merck Sharp & Dohme	sulfate	base	
Neomycin	6W596	Upjohn	"	sulfate	500 mg. = 350 mg. as base
Kanamycin	57K185	Bristol	hemisulfate	base	
Paromomycin	X6873	Parke, Davis	sulfate	sulfate	500 mg. = 360 mg. as base
Erythromycin	8041–687397	Lilly	base	base	
Carbomycin	17K-D528009	Pfizer	phosphate	base	
Oleandomycin	69465	"	"	base	
Spiramycin	FM1176	Merck Sharp & Dohme	sulfate	sulfate	
Bryamycin	56F91	Bristol	base	base	
Leucomycin	C62875	Merrill	base	"	
Streptogramin (900)	552410	Merck Sharp & Dohme	"	"	
MK 111	LD572–146–01	"	"	"	
Novobiocin	0293T	"	sodium	"	

cline the difference may be due to its poorer stability under the conditions of these tests. Streptomycin, neomycin, kanamycin and paromomycin likewise acted very much alike quantitatively; part of the apparently lower activity of neomycin as compared with kanamycin may be due to the fact that the former was made up in terms of the sulfate and concentrations of the latter were calculated as the base. However, concentrations of streptomycin were expressed as the base and those of paromomycin as the sulfate and all of the differences are not significant when the limitations of the method are taken into account.

When compared with the results obtained in this laboratory in previous years, the data shown in Table 2 for

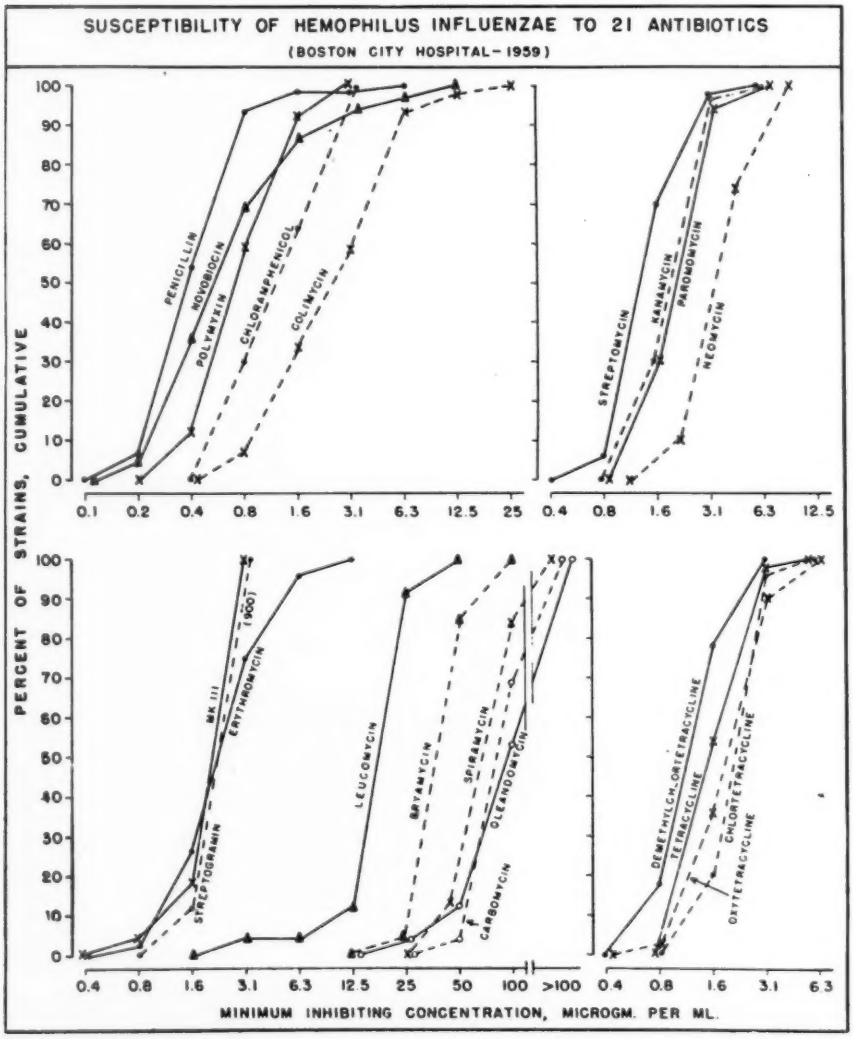

Fig. 1.

TABLE 2.—SUSCEPTIBILITY OF RECENTLY ISOLATED STRAINS OF HEMOPHILUS INFLUENZAE TO 21 ANTIBIOTICS IN VITRO:

TABLE 2.—SUSCEPTIBILITY OF RECENTLY ISOLATED STRAINS OF HEMOPHILUS INFLUENZAE TO 21 ANTIBIOTICS IN VITRO: COMPARISON OF RESULTS OBTAINED IN 1959 WITH THOSE PREVIOUSLY REPORTED FROM THIS LABORATORY

Antibiotic	*Year*	*Number of Strains*	*Minimum Inhibiting Concentration, μg. per ml.*										
			0.2	*0.4*	*0.8*	*1.6*	*3.1*	*6.3*	*12.5*	*25*	*50*	*100*	*>100*
			Percent of Strains										
Penicillin	1959	76	7	47	40	5	0	1					
	1954	90	27	34	20	16	3						
	1949	30	10	13	30	30	13	0	4				
Chloramphenicol	1959	50			30	34	36						
	1954	90		1	66	31	2						
	1949	16			100								
Polymyxin B	1959	76		12	47	33	8						
	1954	11			18	55	27						
	1949	16		6	44	50							
Colimycin	1959	43			7	23	28	35	5	2			
Tetracycline	1959	50				54	44	2					
	1954	90			2	26	72						
Oxytetracycline	1959	50			2	34	54	10					
	1954	90			9	44	47						
Chlortetracycline	1959	50				20	76	4					
	1954	90			2	5	47	46					
	1949	30			47	47	6						
Demethylchlortetracycline	1959	50			18	60	22						
Streptomycin	1959	50			6	64	28	2					
	1954	90				24	49	26	1				
	1949	60	2	8	17	33	33	5	2				
Neomycin	1959	50					10	64	26				
	1954	11					46	46	9				
Kanamycin	1959	50				28	70	2					
Paromomycin	1959	50				28	66	6					
Erythromycin	1959	76			3	24	49	21	4				
	1954	90		1	5	21	57	16					
	1952	18		5	28	50	17						
Carbomycin	1959	26									4	65	31
	1954	11								73	27		
Oleandomycin	1959	26								4	8	43	46
Spiramycin	1959	26									12	62	27
Bryamycin	1959	26								4	81	15	
Leukomycin	1959	26					4	0	8	81	8		
Streptogramin (900)	1959	26				12	88						
MK 111	1959	26			4	12	85						
Novobiocin	1959	30	7	29	33	18	7	3	3				

penicillin, chloramphenicol, 3 of the tetracycline analogues, streptomycin, neomycin, erythromycin and carbomycin indicate that there has been essentially no change in the susceptibility of *H. influenzae* to these antibiotics.

Discussion. It is of interest that, with the exception of bryamycin and some of the antibiotics of the erythromycin group, namely carbomycin, oleandomycin, spiramycin and leukomycin, the antibiotics included in this study inhibited the strains of *H. influenzae* within a fairly narrow range of concentrations that was similar for all of the antibiotics. The minimum inhibiting concentrations of the 5 antibiotics named was 25 μg. per ml. or more for nearly all of the strains, whereas almost all of the same strains were inhibited by 6.3 μg. per ml. or less of all the other antibiotics that were studied.

Similar results were obtained in the previous studies for which these less active agents, except for carbomycin, were not available and therefore had not been included. Four strains of *H. influenzae* previously tested in a separate study of bryamycin, however, were inhibited by concentrations of 25 to 100 μg. per ml. (Jones and Finland[5]). The earlier studies also showed, as did the present one, that a large proportion of the strains (more than one-half of those isolated in 1959 and 1954) were inhibited by 0.4 μg. of penicillin or less per ml., whereas very few or none were inhibited by similarly low concentrations of any of the other antibiotics. Bacitracin, in the 2 previous studies, failed to inhibit any of the strains even in concentrations that were much higher than the maximum used in the present study, and it was therefore omitted this time.

Although susceptibility of an organism, as determined *in vitro*, is of prime importance in the selection of therapy for infections with that organism, the *in vitro* results are not necessarily paralleled by *in vivo* activity. The latter also depends on additional factors such as the distribution of the antibiotics in the body and their rates of excretion and degradation; these factors may vary with different antibiotics, even with some that are as closely related as the tetracycline analogues (Kunin and Finland[6]). The nature of the infected lesion may also contribute to the variable success or failure of different antibiotics. Such difference may account for the fact that cases of *H. influenzae* meningitis generally respond poorly to treatment with penicillin, although occasional cases have had a favorable outcome after therapy with large doses.

It is difficult to compare the results presented here with those reported by others, particularly those based on essentially qualitative methods, employing paper discs or strips impregnated with antibiotics. In one study (Goodier and Parry[4]), for example, paper strips impregnated with 20 units per ml. of penicillin and 50 μg. per ml. of erythromycin showed only 5 and 60% of 16 strains of Hemophilus "sensitive" to these antibiotics, respectively; by contrast nearly all of the strains in the present study were inhibited by one-eighth of these concentrations or less. The present studies do have the virtue that they were carried out by quantitative methods and under conditions which were the same as those employed in previous studies in this laboratory. Comparisons with the results of previous studies should thus have considerable validity, within the limits of error of the methods used.

Summary and Conclusions. Strains of *Hemophilus influenzae* isolated early in 1959, mostly from the respiratory tract of hospitalized patients were tested for *in vitro* susceptibility to 21 antibiotics, including several that had

been used previously for similar tests on strains isolated in 1954 and 1949 and also several groups of related antibiotics.

All of the antibiotics were roughly similar in their activity against these organisms except bryamycin and some of the erythromycin-like antibiotics (carbomycin, oleandomycin, spiramycin and leukomycin) which were much less active, and penicillin which was more active than all the others against more than one-half of the strains.

The results obtained with the strains isolated in 1959 were similar to those obtained with some of the same antibiotics in the 2 earlier studies. In both of those studies all of the strains tested were found to be resistant to bacitracin, which was not included in the present tests.

It may be concluded that there has been no significant change in the antibiotic susceptibility of strains of *H. influenzae* isolated in this hospital during the past 10 years. Most of the newly introduced antibiotics are about as active against *H. influenzae* as those that were previously available, but some of the erythromycin-like antibiotics, namely carbomycin, oleandomycin, spiramycin and leukomycin are much less active. Colimycin appears to be slightly less active than polymyxin B; the 4 tetracycline analogues all have very similar activity and there are only slight, and probably not significant differences in the activity of streptomycin, neomycin, kanamycin and paromomycin against strains of *H. influenzae*.

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SUMMARIO IN INTERLINGUA

Le Susceptibilitate de *Hemophilus influenzae* a 21 Antibioticos *in Vitro*

Racias de *Hemophilus influenzae*, isolate in le prime parte de 1959 principalmente ab le vias respiratori de patientes hospitalisate, esseva testate pro lor susceptibilitate *in vitro* a 21 antibioticos, incluse plures que habeva previemente essite usate in simile tests con racias isolate in 1954 e 1949.

Omne le antibioticos esseva plus o minus simile in lor activitate contra iste organismos, con le exception de bryamycina e plures del antibioticos erythromycinoide, como carbomycina, oleandomycina, spiramycina, e leucomycina, le quales esseva multo minus active, e etiam con le exception de penicillina, le qual esseva multo plus active que le altere agentes contra plus que un medietate del racias.

Le resultatos obtenite con le racias isolate in 1959 esseva simile al resultatos obtenite con partialmente le mesme antibioticos in 2 previe studios. In ambe ille studios il esseva constatate que omne le racias testate esseva resistente contra bacitracina, le qual non esseva includite in le presente tests.

Es justificate concluder que nulle significative alteration ha occurrite in le susceptibilitate antibiotic de racias de *H. influenzae* isolate a iste hospital in le

curso del passate 10 annos. Le majoritate del novemente introducite antibioticos ha plus o minus le mesme activitate contra *H. influenzae* como le antibioticos que esseva previemente disponibile, sed certes del antibioticos erythromycinoide, *i.e.* carbomycina, oleandomycina, spiramycina, e leucomycina, es multo minus active. Colimycina pare esser levemente minus active que polymyxina B. Le 4 analogos de tetracyclina ha omnes un similissime activitate, e il existe solmente leve e probabilemente non significative differentias inter le activitate de streptomycina, neomycina, kanamycina, e paromomycina contra racias de *H. influenzae.*

SUMMARIO IN INTERLINGUA

(See page 41 for original article)

Le Susceptibilitate de Gonococcos pro le Action de Antibioticos e Sulfadiazina Un Studio de Racias Isolate al Hospital del Citate de Boston ab Casos de Acute Urethritis Gonorrheic in Masculos Inter Augusto 1958 e Februario 1959

Racias de gonococcos isolate inter augusto 1958 e februario 1959 esseva testate con respecto a lor sensibilitate pro 20 antibioticos e pro sulfadiazina. Con le exception de penicillina e sulfadiazina, omne le agentes inhibiva omne le racias testate intra un satis restringite scala de concentrationes. Differente antibioticos variava quantitativemente in lor activitate, sed solmente minor differentias esseva notate in le sensibilitate del racias de gonococcos pro le action de chimicamente relationate antibioticos, con le exception del caso del antibioticos relationate a erythromycina. Istos variava grandemente in lor activitate.

Racias isolate ab patientes recentemente tractate con penicillina esseva inter le racias con le plus basse grado de susceptibilitate pro le action de ille antibiotico. Le racias recente esseva quasi omnes altemente o al minus moderatemente sensibile pro le action de sulfadiazina.

Le comparation de iste resultatos con le constatationes reportate in previe annos ab iste laboratorio non revela ulle marcate reduction del sensibilitate de gonococcos pro ulle del antibioticos in uso in recente annos.

SUSCEPTIBILITY OF GONOCOCCI TO ANTIBIOTICS AND SULFADIAZINE

A STUDY OF STRAINS ISOLATED AT BOSTON CITY HOSPITAL FROM CASES OF ACUTE GONORRHEAL URETHRITIS IN MALES, AUGUST, 1958 TO FEBRUARY, 1959*

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There has been much concern recently about the increasing proportion of treatment failures in cases of gonorrhea and about the progressively larger doses of penicillin that seem to be required to effect cures in apparently uncomplicated cases (Cradock-Watson, Shooter and Nicol[1], Curtis and Wilkinson[2], Epstein[4], Reyn, Korner and Bentzon[12], Willcox[19,21]). A few of the reports also present acceptable evidence for some recent decrease in the penicillin susceptibility of strains of *Neisseria gonorrhoeae* isolated from patients which appear to be correlated, to some extent, with the failures of penicillin treatment in the patients (Cradock-Watson, Shooter and Nicol[1], Curtis and Wilkinson[2], Reyn, Korner and Bentzon[12]). In addition, there are also beginning to appear reports of failures from treatment with apparently adequate doses of streptomycin (Curtis and Wilkinson[2], Davey[3], Reyn, Korner and Bentzon[12], Willcox[19]) and broad spectrum and other antibiotics (Epstein[4], Stuhlert[14], Willcox[19]).

Previous studies from this laboratory (Gocke, Wilcox and Finland[5], Love and Finland[10]) had failed to reveal any important increase in resistance of gonococci to any available antibiotics, including penicillin. Moreover, tests with sulfadiazine indicated that there were fewer strains of gonococci resistant to this agent among those isolated and tested in 1953–1954 than among those tested in 1949. The low incidence of sulfonamide-resistant strains among gonococci isolated in recent years has also been confirmed by others (Cradock-Watson, Shooter and Nicol[1], Thayer, Field and Garson[15]). The present report deals with tests of the susceptibility of strains of gonococci isolated late in 1958 and early in 1959

*Aided, in part, by a grant (E–23) from the National Institutes of Health.

from cases of acute male urethritis employing the same methods and antibacterial agents as in the previous studies and additional antibiotics that have become available since the last report.

Materials and Methods. The strains of *N. gonorrhoeae* included in this study were all isolated in pure culture and identified by Mrs. Helen Trousdale of the genitourinary clinic of the Boston City Hospital from purulent urethral discharge of male patients between August, 1958, and February, 1959. Most of the strains were obtained during the initial visit, but some of them were isolated within a few days following recent treatment with a single dose of 600,000 units of procaine penicillin, which was the standard initial treatment in such cases during this period. An effort was made to determine whether there had been any recent re-exposures in the cases of apparent failure.

The media and the methods used in carrying out the sensitivity tests were the same as in the previous study (Love and Finland[10]). Details concerning the antibiotics used, which were also employed in a study of strains of *Hemophilus influenzae*, are listed elsewhere (Hirsch and Finland[6]). All of the available strains were tested with penicillin, and varying numbers were tested with other antibiotics. The tests with related antibiotics (the tetracycline analogues, 2 polymyxins, the erythromycin-like antibiotics and those similar to neomycin) were carried out simultaneously. These strains were not tested with bacitracin or novobiocin; however, results obtained with a few strains tested with the latter by the same method in 1955 (Jones, Nichols and Finland[7]) are included.

Results. The results of all the tests are summarized in Table 1 and are represented graphically in Fig. 1. From the range of concentrations that inhibited these strains, as shown in the table, and from the slope and shape of the curves representing the cumulative percent of strains inhibited by increasing concentrations of the individual agents it is seen that the inhibitory action of each of these drugs was moderately uniform for most of the strains. Nearly all of the strains tested with any one of these agents were inhibited by concentrations of that agent which varied only 2- or 4-fold from the median. Penicillin and sulfadiazine were exceptions; the inhibiting concentrations of each of these 2 agents varied over more than a 200-fold range. However, there were fairly wide quantitative differences in the action of the individual antibiotics; these are reflected in the different positions of the corresponding curves from left to right in Fig. 1. Penicillin, erythromycin and the tetracyclines were the most active among the agents in common use, whereas polymyxin and the similar new antibiotic, colimycin, were the least active, weight for weight. All but 1 of the 10 strains that were tested with novobiocin in 1955 were inhibited by 3.1 or 6.3 μg. per ml. (Jones, Nichols and Finland[7]) similar concentrations of bryamycin inhibited the recent strains.

Table 1 and Fig. 1 also show that the 4 tetracycline analogues were about equally active against the strains with which they were tested. The new analogue, demethylchlortetracycline (Kunin and Finland[8], McCormick *et al.*[11]), appeared to be slightly more active, and chlortetracycline slightly less active than both tetracycline and oxytetracycline, which were about equally active; 85% of the individual strains were inhibited by concentrations of demethylchlortetracycline which were slightly lower than, or at least the same as, the most active of the other 3 analogues.

The 4 antibiotics, streptomycin, neomycin, kanamycin and paromomycin, which are related to some extent in their chemical structure and activity (Kunin *et al.*[9]), showed only minor differences in their activity against the gonococcal strains with which they were tested. Part of the apparently lower activities of neomycin and paromomycin is related to the fact that

TABLE 1.—*IN VITRO* SUSCEPTIBILITY OF RECENTLY ISOLATED STRAINS OF NEISSERIA GONORRHOEAE TO 21 ANTIBIOTICS AND SULFADIAZINE

Antibiotic	*Number of Strains*	*Minimum Inhibiting Concentration, μg. per ml.*											
		≤0.001	0.002	0.005	0.01	0.02	0.04	0.1	0.2	0.4	0.8	1.6	3.1	6.3
		Percent of Strains												
Penicillin	157	5.7	12.7	20.4	25.5	10.8	15.9	8.3	0.6					
Tetracycline	132						0.8	7.6	30.3	45.5	14.4	1.5		
Oxytetracycline	132						1.5	11.4	34.8	44.7	6.1	1.5		
Chlortetracycline	132						2.3	4.5	18.2	41.7	26.5	5.3	1.5	
Demethylchlortetracycline	132					0.8	4.5	20.5	39.4	31.8	3.0			
Chloramphenicol	48										42.	27.	31.	
Erythromycin	132					6.1	26.5	56.1	10.6	0.8				
Carbomycin	36									6.	8.	31.	44.	11.
Oleandomycin	59							3.	0	3.	32.	41.	20.	
Spiramycin	36									3.	44.	44.	8.	
Leukomycin	36						3.	3.	14.	58.	22.			
Streptogramin (900)	36				3.	22.	42.	33.						
MK 111	36					3.	19.	64.	14.					
Bryamycin	38												34.	66.
Novobiocin*	11											9.	36.	55.

Antibiotic	*Number of Strains*	*Minimum Inhibiting Concentration, μg. per ml.*									
		0.4	0.8	1.6	3.1	6.3	12.5	25	50	100	>100
		Percent of Strains									
Streptomycin	131			1.5	16.0	32.8	42.7	6.9			
Neomycin	23						30.	30.	39.		
Kanamycin	23				4.	9.	87.				
Paromomycin	23						52.	48.			
Polymyxin B	37							5.	14.	41.	41.
Colimycin	36							3.	17.	39.	42.
Sulfadiazine	132	0.8	1.5	1.5	3.0	11.4	22.7	31.8	16.7	9.8	0.8

*Tested in 1955.

For a similar tabulation of the results of tests done in this laboratory in 1954 and earlier with 11 of these drugs and with bacitracin, see Love and Finland.[10]

they are expressed as the sulfate whereas those of the other two are expressed in terms of the base.

The action of colimycin* was the same as that of polymyxin B against nearly all strains.

and leukomycin was intermediate in its activity. Erythromycin was at least 16 times more active than oleandomycin or spiramycin against nearly all of the strains. Streptogramin and MK 111, which is a very similar antibiotic, were

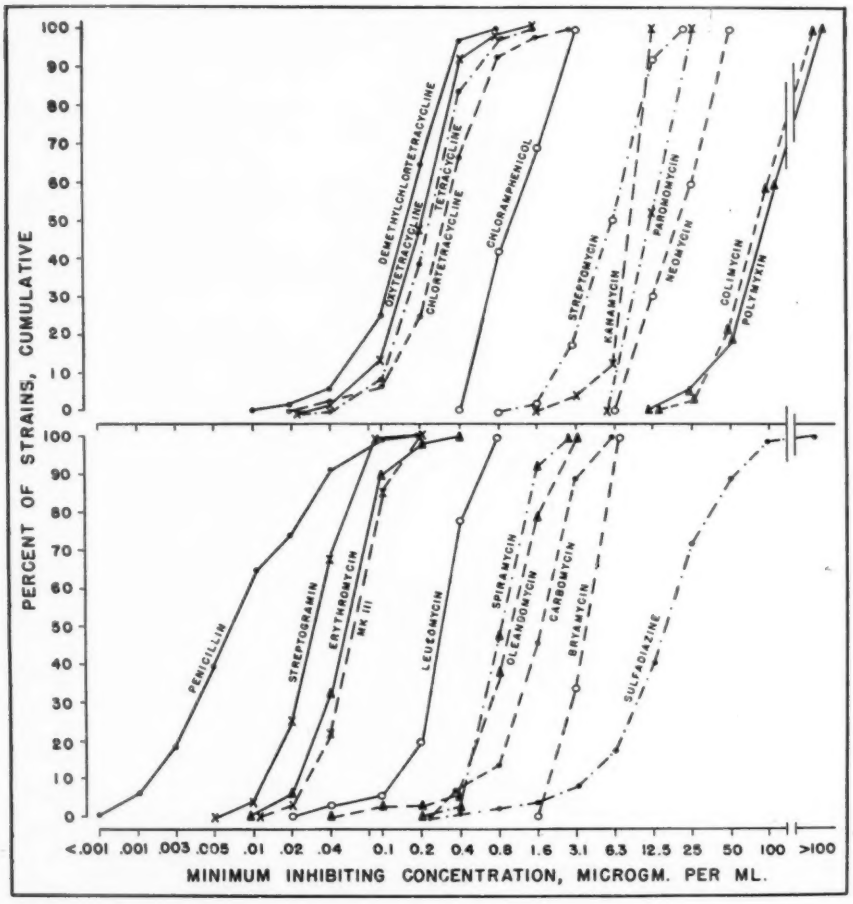

Fig. 1.—*In vitro* susceptibility of *Neisseria gonorrhoeae* to 20 antibiotics and sulfadiazine; strains isolated at Boston City Hospital, August, 1958 to February, 1959.

Erythromycin was the most active and carbomycin the least active of the erythromycin (macrolide) group of antibiotics; oleandomycin and spiramycin were about equally active but only slightly more active than carbomycin, about equally active, their inhibiting concentrations being about the same as that of erythromycin.

COMPARISONS WITH RESULTS OBTAINED IN PREVIOUS YEARS. The results of tests done in this laboratory in previous

*Recently given the generic name colistin.

years (Love and Finland[10]) with some of the same antibiotics that were available at the time are shown in Figs. 2, 3 and 4 together with the corresponding results obtained with the recent strains. The curves shown for the tetracyclines in Fig. 3, and for streptomycin, chloramphenicol and erythromycin in Fig. 4 all have one important feature in common, namely, that the curves in each panel, representing the activity

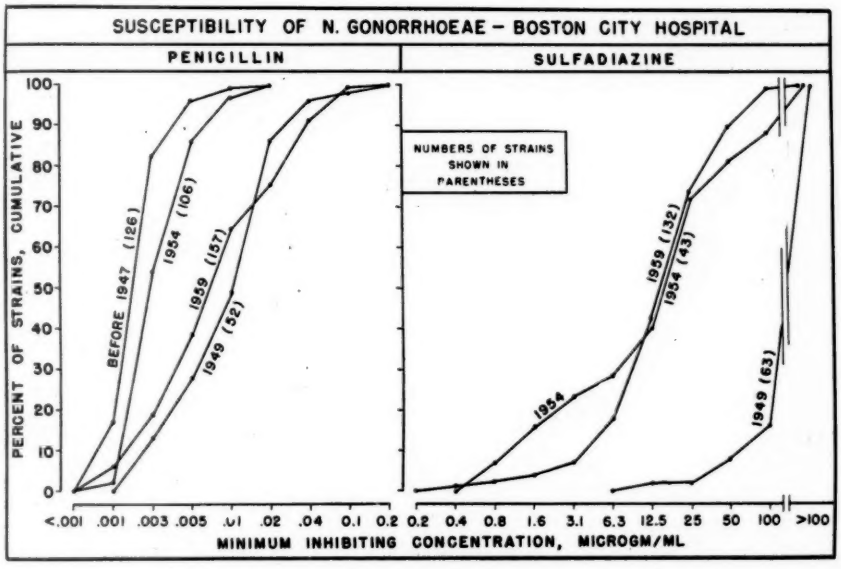

Fig. 2

of each of the individual antibiotics in different years, have the same shape and slope and extend over a similar range of concentrations. In the case of the tetracyclines and erythromycin, the curves are slightly to the left implying that the recent strains were apparently more sensitive, whereas the 1959 curves for streptomycin and chloramphenicol are to the right implying that the recent strains were somewhat less sensitive. The differences in each instance are generally only 2-fold and occasionally 4-fold and are probably not significant; presumably they represent uncontrollable technical differences in the tests as done and interpreted in the different years in spite of the attempts to maintain uniform conditions and indeed are within the limits of error of the method.

The situation with respect to penicillin and sulfadiazine, as shown in Fig. 2, is different. The curve representing the activity of sulfadiazine against the recent strains differs from that representing the 1954 strains, the former showing a smaller percentage inhibited in the more sensitive range (6.3 μg. per ml. or less) and a greater proportion requiring moderately high concentrations (50 or 100 μg. per ml.). On the other hand, whereas 5 of the 43 strains isolated and tested in 1954 were not inhibited by 100 μg. per ml. of sulfadiazine (10 mg. per 100 ml.), only one among the 132 recent strains grew uninhibited in this concentration. The strains of both these series, however,

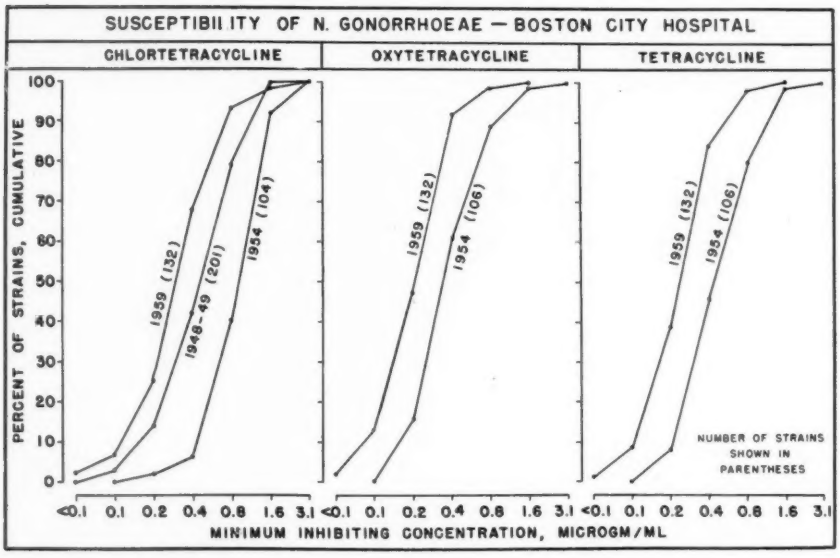
SUSCEPTIBILITY OF N. GONORRHOEAE — BOSTON CITY HOSPITAL
CHLORTETRACYCLINE
OXYTETRACYCLINE
TETRACYCLINE
PERCENT OF STRAINS, CUMULATIVE
1959 (132)
1948-49 (201)
1954 (104)
1959 (132)
1954 (106)
1959 (132)
1954 (106)
NUMBER OF STRAINS SHOWN IN PARENTHESES
<0.1 0.1 0.2 0.4 0.8 1.6 3.1
MINIMUM INHIBITING CONCENTRATION, MICROGM/ML

Fig. 3

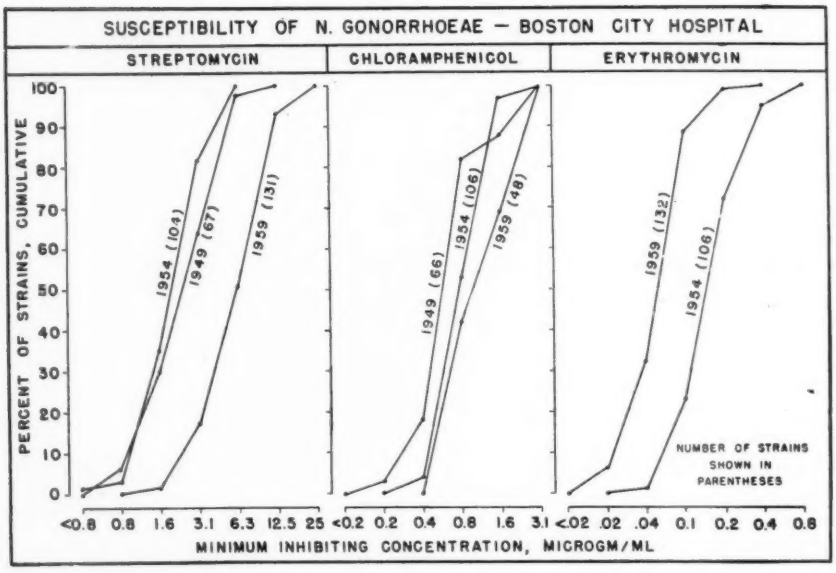
SUSCEPTIBILITY OF N. GONORRHOEAE — BOSTON CITY HOSPITAL
STREPTOMYCIN
CHLORAMPHENICOL
ERYTHROMYCIN
PERCENT OF STRAINS, CUMULATIVE
1954 (104)
1949 (67)
1959 (131)
1949 (66)
1954 (106)
1959 (48)
1959 (132)
1954 (106)
NUMBER OF STRAINS SHOWN IN PARENTHESES
<0.8 0.8 1.6 3.1 6.3 12.5 25
<0.2 0.2 0.4 0.8 1.6 3.1
<.02 .02 .04 0.1 0.2 0.4 0.8
MINIMUM INHIBITING CONCENTRATION, MICROGM/ML

Fig. 4

were much more sensitive to sulfadiazine than those isolated and tested in the same manner in 1949, when the great majority of the strains were resistant to 100 μg. per ml. of this drug.

somewhat greater proportion in the less sensitive range (0.04 to 0.2 μg. per ml.) among the recent isolates. In a previous comparison of strains isolated during post-treatment follow-up visits

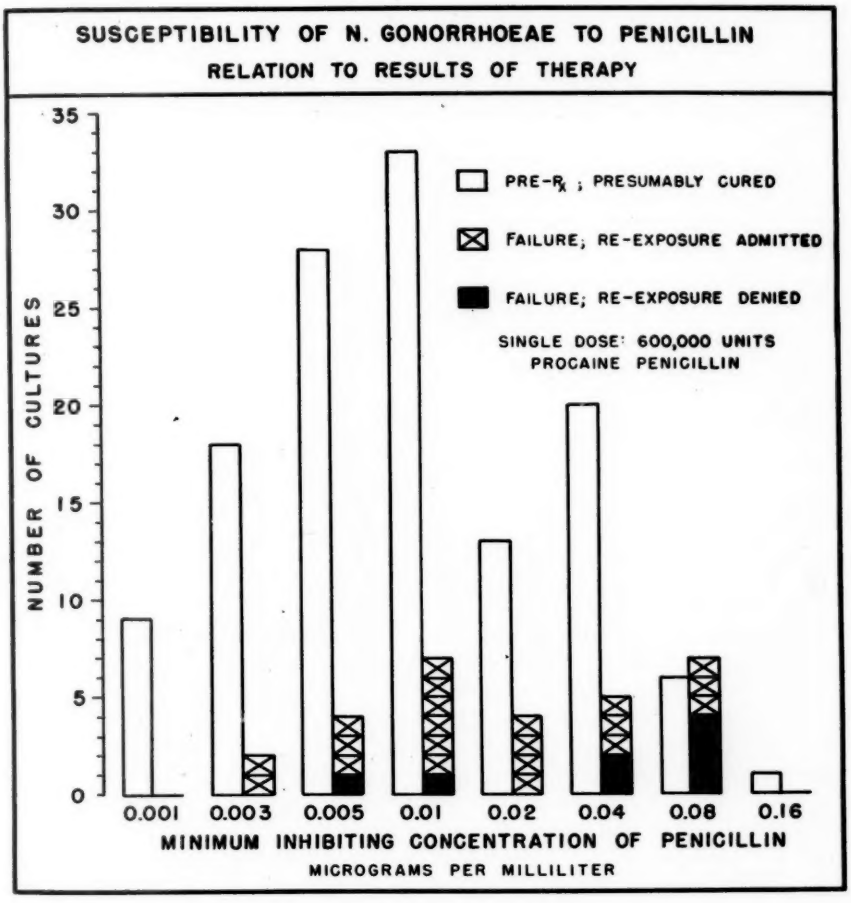

Fig. 5

The results obtained with penicillin at 4 different times are depicted in the left panel of Fig. 2. The findings in 1954 quantitatively resemble those obtained with the strains isolated and tested in 1947 or earlier, whereas the 1959 curve, representing the most recent strains, resembles that of 1949, except for a

(that is, from presumed penicillin treatment failures) with primary isolates obtained pretreatment, no differences in susceptibility were noted (Love and Finland[10]). In the present series, however, the strains obtained in follow-up visits from penicillin treatment failures included a larger proportion of the least

sensitive ones. This is shown in Fig. 5; here it is seen that 6 of the 8 strains obtained after treatment from patients who denied re-exposure, were inhibited by 0.04 or 0.08 μg. per ml., whereas most of the pretreatment strains from the patients who were presumably cured, were inhibited by lower concentrations. The 21 post-treatment strains that were obtained from patients who admitted re-exposures appeared to be more widely distributed with only a slightly greater proportion in the less sensitive range as compared with primary isolates from patients who were cured.

Discussion. The data presented here concerning penicillin, meager though they be, offer the first indication from this clinic of a possible decrease in susceptibility of gonococci from cases of urethritis that may be correlated with treatment failures. In these respects, findings from Copenhagen reported by Reyn, Korner and Bentzon[12], who used assay methods that are comparable to those employed here, are the most convincing of any that have been presented to date. These authors tested 90 strains isolated in 1944 and preserved by lyophilization at about the same time and by the same method with which they tested 2 other groups of strains isolated in 1957. One of the latter consisted of 103 strains isolated from material submitted routinely for diagnosis, the other included 100 strains specifically submitted for sensitivity testing and presumably involved some difficulty in therapy of the patients. The 1944 strains showed a "normal" distribution pattern of sensitivity to penicillin, with a peak corresponding to 0.01 μg. per ml. and a range from 0.0038 to 0.024 μg. per ml. (6-fold). Among the 1957 "diagnostic" strains there were 2 peaks, one at 0.01 and the other 0.2 μg. per ml., with a range from 0.0055 to 0.43 μg. per ml. (80-fold); the peak in the high concentration range was only a low one and only a small proportion of these strains required more than 0.1 μg. per ml. By contrast, the 100 strains submitted for sensitivity tests in 1957 had a higher peak at 0.2 μg. per ml. and a considerably larger proportion of the strains in the higher concentration range above 0.1 μg. per ml. One strain in the "diagnostic" group and 3 in the "sensitivity" group were resistant to streptomycin and there was a suggestion of a change in the normal distribution pattern similar to that observed with penicillin but within the same narrow range. No definite trend with respect to susceptibility to tetracycline was noted among any of these strains.

In another report Curtis and Wilkinson[2], using different media in a tube-dilution test, reported that 19.5% of 302 pretreatment strains isolated in 1957 required 0.125 to 0.5 unit (0.08 to 0.3 μg.) per ml. for inhibition. Failures of treatment with 300,000 units of procaine penicillin (or penicillin in 2% aluminum monostearate) occurred in the patients from whom these strains were isolated and none among those whose pretreatment strains were inhibited by less than 0.03 unit (0.02 μg.) per ml. Cradock-Watson, Shooter and Nicol[1] also tested 200 strains isolated between August, 1957, and February, 1958, in 2 large London hospitals; 19% of these strains were inhibited by penicillin in concentrations of 0.128 to 0.512 unit (0.08 to 0.32 μg.) per ml. Relapses after treatment occurred in 34% of the patients from whom these strains were isolated as compared with 7% of patients whose strains were inhibited by smaller concentrations. All the strains were inhibited by 16 μg. per ml. or less of streptomycin and only 1 required more than 8 mg. per 100 ml. (80 μg. per ml.) of sulfathiazole.

Recently Barile, Van Zee and Yaguchi (Antibiotics & Chemotherapy, **6**, 470, 1959) reported a series of 165 patients with gonorrhea treated in a military hospital in Japan; 7 failed to respond to the intramuscular administration of 600,000 units of procaine penicillin G daily for 3 days. The 7 strains isolated from these patients were inhibited by 0.25 to 1.0 unit per ml. of penicillin as compared with a control strain from a cured patient which was sensitive to 0.03 unit per ml. (1 unit = 0.6 μg./ml.).

Thayer, Field and Garson[15] reported that all of 43 strains of gonococci isolated in North Carolina were inhibited by 200 μg. per ml. or less of either sulfadiazine or sulfamethoxypyridazine and only 2 of their strains required this large amount. These findings and those of Cradock-Watson, Shooter and Nicol[1] confirm the findings in this study and previous observations made in this laboratory in 1954 (Love and Finland[10]), that nearly all strains of gonococci now are sensitive to sulfonamides. This, however, should not be considered as adequate reason for recommending a return to sulfonamide therapy of gonococcal infections both because of the likelihood of rapidly reestablishing the sulfonamide-resistant strains and because this therapy is less reliable and has never been nearly as successful as modern antibiotic treatment when properly used. Moreover, the value of combined therapy of a sulfonamide with penicillin or another antibiotic has never been proved and indeed, has not yet been given an adequate clinical trial.

The possible reasons for failures of penicillin treatment in many cases of gonorrhea have been discussed recently by several authors (Epstein[4], Reyn, Korner and Bentzon[12], Schamberg[13], Stuhlert[14], Thayer *et al.*[16,17,18], Willcox[19,20,21]). Some of the clinical failures may be related to errors in diagnosis, mixed or complicated infections, and possibly to the use of repository preparations that yield relatively low concentrations, particularly benzathine penicillin (Willcox[21]). As mentioned earlier, there have been very few instances (limited chiefly to the reports cited above) in which such failures have been clearly correlated with decreased susceptibility of the causative gonococcal strain to antibiotics. In dealing with the failures, proper diagnosis, both clinical and bacteriological, are essential, and a search must be made for other causes of the failure. In general it is fair to say that there has been no great change in the susceptibility of gonococci to penicillin in this area. The changes observed here and elsewhere are still relatively small; nevertheless they are of sufficient magnitude to necessitate a significant adjustment in the dosage schedules.

It is important, however, and particularly for the genitourinary surgeon, to avoid manipulation and instrumentation of the genitourinary tract in cases of treatment failures of gonorrhea, because, in spite of any decrease in the susceptibility of some strains of gonococci to penicillin, they are still among the most sensitive ones we have to deal with, and any infection that might be introduced during the manipulation is almost certain to involve organisms that are less susceptible to antibiotics and hence likely to be much more difficult to cure by antibacterial therapy.

In interpreting the relative *in vitro* activity of different antibiotics for therapeutic purposes, it is important to bear in mind that other factors may play an equal or even more important role in their clinical effectiveness. Among these factors are the absorption, distribution and excretion of the drugs, particularly their effective concentrations in the urine and their abil-

ity to penetrate into infected areas. Few, if any of the antibiotics may be expected to act on intracellular organisms (Thayer *et al.*[17,18]), and this may be of considerable importance. The presence of mixed infections, particularly with penicillinase producing staphylococci may be another important factor in the failure to eliminate the gonococcus; in such cases, it is essential to include another agent directed specifically against the secondary invaders.

Summary and Conclusions. Strains of gonococci isolated in August, 1958, to February, 1959, were tested for sensitivity to 20 antibiotics and sulfadiazine. With the exception of penicillin and sulfadiazine, each of the agents inhibited all of the strains tested within a fairly narrow range of concentrations. Different antibiotics varied quantitatively in their activity, but there were only minor differences in the susceptibility of the strains to chemically related antibiotics except in the case of those related to erythromycin which varied quite widely in their activity.

Strains isolated from patients soon after treatment with penicillin were among the least susceptible to that antibiotic. The recent strains were nearly all highly or moderately susceptible to sulfadiazine.

Comparisons of these results with the findings reported in previous years from this laboratory show no evidence of any large decrease in susceptibility of gonococci to any of the antibiotics that have been in use during the past few years.

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(Interlingua on page 40)

INFLUENCE OF ERYTHROCYTES ON THE COAGULATION OF BLOOD*

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Although Wooldridge[44] in 1886 and, later, Barratt and Yorke[2] had shown experimentally that the stroma of erythrocytes possessed clotting activity, the firmly entrenched concept that the platelets are the only cellular constituents which participate in the physiological coagulation of the blood has hampered impartial consideration of the possible role that erythrocytes might have in the clotting reaction and in hemostasis. A few investigators, particularly Shinowara[41] and Gollub[14], observed that the erythrocytes and other blood cells contain factors that influence blood coagulation. They interpreted this activity to be thromboplastic in nature. Georgatsos, Hussey and I[13,28] showed that erythrocytes contain a clotting factor resembling that of platelets but differing from the latter by being not only more potent but also by possessing various activities lacking in the platelets. In the present communication, the results of further studies concerning the origin of the clotting agent in erythrocytes are presented, and the possible role of the red cells in the clotting reaction is discussed.

Materials and Methods. COLLECTION OF BLOOD. Blood obtained by venipuncture with a silicone-coated syringe and needle was immediately transferred to glass test tubes similarly coated and these were placed in an ice bath.

PREPARATION OF PLATELET-POOR NATIVE PLASMA. The blood which was chilled for 20 to 30 minutes in an ice bath was centrifuged for 30 minutes at 12,000 rpm. in a Servall Superspeed centrifuge in a cold room kept at 4° to 6° C. The plasma was immediately removed with care so that the platelet layer was not disturbed. Such plasmas rarely have a platelet count greater than 1000 per c.mm.

PREPARATION OF PLATELET-RICH PLASMA. Blood kept in a silicone-coated test tube was allowed to sediment in an ice bath. As was observed previously (Quick, Shanberge and Stefanini[32]), the platelet count of such plasma is considerably higher than of whole blood.

PROTHROMBIN CONSUMPTION TIME (BASIC). One ml. of plasma or whole blood was transferred to a glass test tube 13 × 100 mm. and placed in a water bath at 37° C. Exactly 15 minutes after a solid clot formed, the tubes were centrifuged for 1 minute or, in the case of the plasma, the clot was removed by wrapping it on a wooden applicator. After the separation of the serum from the clot, the test tube was incubated 45 minutes more. The prothrombin activity of the serum was determined as follows: 0.1 ml. of the serum was blown into a mixture of 0.1 ml. of thromboplastin reagent (prepared from acetone-dehydrated rabbit brain), 0.1 ml. of 0.02M calcium chloride, and 0.1 ml. of fresh oxalated rabbit plasma adsorbed with $Ca_3(PO_4)_2$. The time from the addition of the serum to the formation of a clot was accurately determined with a stop watch. When the consumption of prothrombin is very defective, the serum prothrombin time

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is 8 to 9 seconds; the abnormal range is from 8 to 14 seconds.

NORMAL AGED SERUM. Normal blood was clotted at 37° C. and 15 minutes after clotting, the serum was separated from the clot by centrifugation. The serum was incubated for an hour at 37° C. and an additional 24 hours at 4° C. A number of sera thus prepared were pooled, divided into 1 ml. portions, and stored at −20° C.

HEMOLYSATE REAGENT. Erythrocytes, from which the platelets and leukocytes were removed by differential centrifugation and repeated washing with saline solution, were suspended in physiological saline solution and frozen at −20° C. On thawing, complete hemolysis was obtained. The exact details have been previously described (Quick[25]).

TABLE 1.—THE INFLUENCE OF GLASS AND SILICONE SURFACES ON THE PROTHROMBIN CONSUMPTION TIME IN THE CLOTTING OF BLOOD AND OF PLATELET-RICH AND PLATELET-POOR PLASMA

	Prothrombin Consumption Time (sec.)					
	Whole Blood		*Platelet-rich Plasma*		*Platelet-poor Plasma*	
	Silicone	*Glass*	*Silicone*	*Glass*	*Silicone*	*Glass*
1 ml. of blood or plasma:	12	24	12	23	12	9
after freezing and thawing	39	120	12	42	12	9
0.03 ml. aged serum added	27	31	26	44	11	9
0.1 ml. hemolysate added	80	107	35	100	37	75
0.1 ml. hemolysate added 0.03 ml. aged serum added	120	200	88	140	67	160

Results. INFLUENCE OF SURFACE ON THE PROTHROMBIN CONSUMPTION OF PLATELET-POOR AND PLATELET-RICH PLASMA. No significant consumption of prothrombin occurs when either platelet-rich or platelet-poor plasma is clotted in a silicone-coated test tube (Table 1). There is no detectable consumption of prothrombin when platelet-poor plasma is clotted in either silicone or glass but the serum prothrombin time in the latter is 8 to 9 seconds, whereas in silicone it is 12 seconds. A probable explanation (Quick[26]) is that human plasma contains most of its prothrombin in an inactive form which becomes activated by contact with glass, hence, the decrease of the serum prothrombin time from 12 to 8 or 9 seconds.

Mixing platelet-poor plasma with a small amount of aged serum before clotting occurs has no effect on the prothrombin consumption time either in glass or silicone, but by adding serum to platelet-rich plasma the utilization of prothrombin is greatly increased even in a silicone-coated test tube and more in glass. Apparently, serum contains an agent which functions in bringing the platelets into the clotting reaction. Since prothrombin consumption occurs when platelet-rich plasma is clotted in glass, it seems fairly evident that contact with glass is instrumental in producing the serum agent that is needed for the utilization of platelets. The action is chemical rather than physical because freezing and thawing the plasma, which has a disintegrating action on platelets, has little or no effect on prothrombin consumption.

The addition of a standardized extract of erythrocytes (hemolysate) to

native plasma, whether platelet-poor or -rich, brings about a significant consumption of prothrombin. Since the effect is not essentially greater in platelet-rich plasma than in platelet-poor, it seems logical to conclude that the action of hemolysate is independent of the platelets. When whole blood is clotted in a silicone-coated test tube, the prothrombin consumption time is 12 seconds, provided care is taken to avoid any contact with glass or vigorous agitation that might injure the erythrocyte mechanically. Even a minor deviation from this meticulous technique may cause a consumption time of 20 seconds or more. In a platelet-rich plasma, a prothrombin consumption time of 12 seconds is also obtained when the clotting is carried out in silicone, even when the platelets are mechanically disintegrated by repeated freezing and thawing.

EFFECT OF SURFACE ON PLATELET-POOR PLASMA MEASURED BY THE PROTHROMBIN CONSUMPTION TIME AFTER MIXING WITH NORMAL BLOOD. It was observed that when whole blood and the platelet-poor plasma from the same blood (neither of which had been exposed to any surface other than silicone) were mixed in equal volume and clotted in glass, a surprisingly poor consumption of prothrombin occurred (Table 2). The values are distinctly lower than those of the corresponding whole blood or of a mixture of platelet-rich and platelet-poor plasmas.

TABLE 2.—THE EFFECT OF MIXING WHOLE BLOOD WITH AN EQUAL VOLUME OF ITS OWN PLATELET-POOR PLASMA ON THE PROTHROMBIN CONSUMPTION

	*Prothrombin Consumption Time (sec.)** *Subjects*									
	1		*2*		*3*		*4*		*5*	
	Mid.M.	*M.*	*Mid.M.*	*M.*	*Mid.M.*	*M.*	*Mid.M.*	*M.*	*Mid.M*	*M.*
Whole blood 1 ml.	19	27	29	21	36	30	17	34	28	25
Whole blood 0.5 ml. Platelet-poor plasma 0.5 ml.	10	12	15	17	17	13	15	14	16	15
Platelet-rich plasma 0.5 ml. Platelet-poor plasma 0.5 ml.	22	30	20	32	32	38	40	33	28	39
Platelet count $\times 10^3$	366	320	290	234	200	250	339	350	205	258

*Neither the blood nor the plasmas came in contact with glass until they were mixed. The clotting was carried out in glass test tubes.
The subjects were normal young women. Blood was taken in the mid-menstrual (Mid.M.) and menstrual (M.) periods.

To determine whether glass surface or freezing and thawing changed the activity of the plasma, the experiments recorded in Table 3 were carried out. When plasma was in contact only with silicone surface, little consumption of prothrombin resulted when it was mixed with whole blood and the clotting carried out in a silicone-coated test tube. Freezing and thawing the plasma did not alter the results. When the plasma was exposed to glass for 5

minutes, or frozen and thawed in glass and then mixed with an equal volume of blood and the coagulation allowed to proceed in a silicone-coated tube, a high consumption of prothrombin resulted.

TABLE 3.—EFFECT OF EXPOSING PLASMA TO GLASS AND OF FREEZING AND THAWING IT BEFORE MIXING WITH WHOLE BLOOD ON THE PROTHROMBIN CONSUMPTION TIME

	*Prothrombin Consumption Time (sec.)**
Plasma not exposed to glass	13.5
Plasma exposed to glass for 5 min.	30.0
Plasma frozen and thawed in silicone	13.5
Plasma frozen and thawed in glass	30

*The clotting was carried out in a glass test tube. The platelet-poor plasma (0.5 ml.) was mixed with an equal volume of whole blood.

Since exposure to glass appeared to effect the activation of platelet-poor plasma in the above experiments, the relation of duration of contact to activation as measured by the utilization of prothrombin was studied (Fig. 1). The activation of plasma by glass is rapid and usually attains its maximum value in 4 to 5 minutes at 25° C. At ice bath temperature (2° C.), the activation is somewhat slower.

DIFFERENCE IN THE PROTHROMBIN CONSUMPTION OF WHOLE BLOOD AND PLATELET-RICH PLASMA IN MILD HEMOPHILIA. In the study of mild hemophilia, results such as recorded in Table 4 are frequently observed (Quick and Hussey[29]). When blood from a mild hemophiliac is clotted under standardized conditions, as outlined by the author, the prothrombin consumption time is often normal, whereas when the test is done on platelet-rich plasma, values in the abnormal range of 8 to 14 seconds are obtained. It is to be noted that the addition of hemolysate to plasma from a mild hemophiliac greatly increases the prothrombin consumption time, whereas when mixed with plasma from a severe hemophiliac, the prothrombin consumption time is exceedingly short.

Discussion. Early in the study of blood coagulation, the influence of contact with foreign surface on the coagulation of blood was observed. Lister[19], in 1863, postulated that this contact induced a "mutual reaction between its solid and fluid constituents in which the corpuscles impart to the liquor sanguinis a disposition to coagulate." Freund[12] and, somewhat later, Bordet and Gengou[7] observed that nonwettable surfaces, such as vaseline and paraffin, delayed coagulation. Although

TABLE 4.—THE PROTHROMBIN CONSUMPTION TIME OF WHOLE BLOOD AND OF PLATELET-RICH PLASMA IN MILD AND SEVERE HEMOPHILIA*

	Subjects		
	A (mild)	*B (mild)*	*C (severe)*
Clotting time (min.)	11	10	60
Prothrombin consumption time (sec.)			
Platelet-rich plasma	12	13	8
Whole blood	15	21	8
With added hemolysate	40	45	8

*The diagnosis of classical hemophilia was made on the clinical history of bleeding, the hereditary pattern, the low concentration of thromboplastinogen, and the defective thromboplastin generation localized in the plasma by the test of Biggs and Douglas.

Bordet and Gengou[8] reported that clotting occurred in what they assumed was plasma, free of cellular elements, the prevailing theory at the beginning of the century held that clotting was initiated by the liberation of a factor

from a cellular element (leukocytes). In fact, in 1903, Dastre[11] called the concept the classical theory. It was tacitly accepted by many investigators that the thrombokinase of the well-known theory of Morawitz was obtained from the lysis of platelets.

In opposition to this concept, a few investigators, Nolf[22], Lenggenhager[18], and Lozner and Taylor[20], held that cell-free plasma contained all the factors essential for coagulation. In 1947 Brinkhous[9] postulated that plasma contained a thrombocytolysin which was responsible for the liberation of thromboplastin from platelets. Quick[24] and, somewhat later, Patton, Ware and Seegers[23] found no significant amount of thromboplastin in platelets. The development of the prothrombin consumption test in 1947 marked a significant advance because it established that neither the platelets nor the plasma furnished thromboplastin, but that this agent was generated from the interaction of a platelet factor and a constituent designated as thromboplastinogen to distinguish it from the antihemophilic globulin which Lozner and Taylor stated was activated by contact with a foreign surface alone. The work of Schulman and Smith[38], Aggeler *et al.*[1], and Biggs and Douglas[5] and their associates established that a third factor (PTC or Christmas factor) was essential for the production of intrinsic thromboplastin. This concept found experimental support from the development of the thromboplastin generation test of Biggs and Douglas[3].

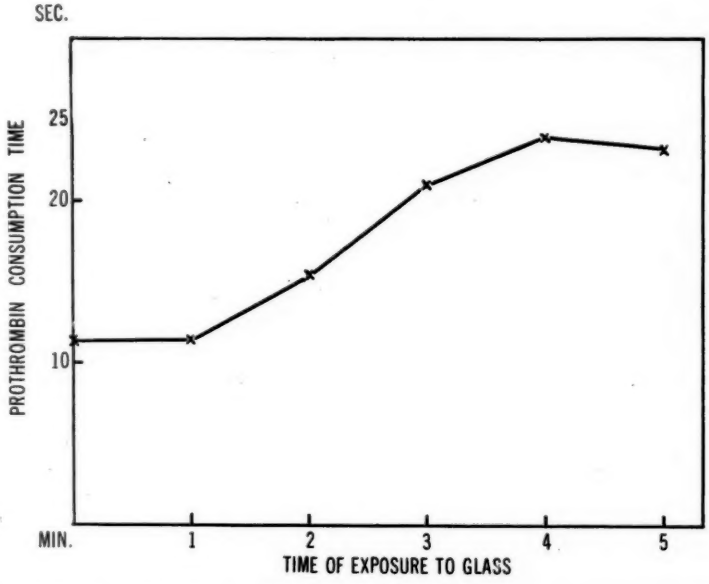

Fig. 1.—The influence of contact with glass on platelet-poor plasma as measured by the prothrombin consumption time after mixing with an equal volume of whole blood.

Despite these developments, the question regarding the initiation of the clotting reactions remained unanswered. Hartmann, Conley and Lalley[15] found that clotting did occur in native plasma depleted of platelets when in contact with glass, but in confirmation of Quick's finding, they observed that

the consumption of prothrombin was low and increased with the platelet count. Later, Conley, Hartmann and Morse[10] attributed the clotting of platelet-free plasma to a specific precursor which could, like platelets, initiate coagulation. They acknowledged that both were essential for normal coagulation. Patton, Ware and Seegers[23] stated that they could find no "evidence of a soluble factor in plasma that could initiate clotting independent of platelet action." Quick and Epstein[27] found that little consumption of prothrombin occurred when a platelet extract was added to native plasma if the coagulation occurred in a silicone-coated tube, unless the plasma was previously exposed to glass. Later, it was shown that consumption of prothrombin did occur in silicone if a small amount of thrombin was added to the mixture of platelet extract and plasma (Quick, Hussey and Epstein[31]). This suggested that thromboplastinogen required activation by thrombin before it could react with platelets to form thromboplastin. Ratnoff and Conley[35] also concluded that their results "suggest that the clot-promoting effect of glass is due to its action on the precursor of the plasma thromboplastic factor which is deficient in hemophilia."

Biggs, Douglas and Macfarlane[4] obtained evidence suggesting that the Christmas factor (PTC) and platelets are activated by contact with foreign surface and they suggest the possibility that this may be the initial step in coagulation. Rapaport, Aas and Owren[33] arrived at a similar conclusion. Other investigators also confirmed the finding that PTC (Christmas factor or factor IX) is in an inactive state (Koller, Baer and Geiger[17], Quick and Hussey[30]).

The probability that the plasma factor, which, on activation initiates coagulation, is neither thromboplastinogen nor PTC but a separate factor is suggested by the studies of Shafrir and de Vries[40] and of Margolis[21]. The latter pointed out the similarity of this agent to the one lacking in the Hageman defect described by Ratnoff and Colopy[34]. Ratnoff and Rosenblum[36] have reported findings supporting the concept that the action of glass in accelerating clotting requires the Hageman factor. Biggs *et al.*[6] have concluded that 2 factors are involved in the activation by glass: PTA, the factor lacking in the condition described by Rosenthal, Dreskin and Rosenthal[37], and the Hageman factor. Similar views are expressed by Waaler[43] in his comprehensive summary of the problem. Soulier, Wartelle and Menache[42] conclude that the activation of the antihemophilia B factor (PTC) by glass is actually mediated through the activation of the Hageman factor.

Although the various findings, views and conclusions appear *a priore* to be disjointed and often contradictory, a careful analysis of the data discloses a gradual clarification and the beginning of a unified concept. The results obtained in the present investigation should contribute further to a better understanding of the problem.

It has become well established that a plasma factor becomes activated by contact with glass but in the absence of platelets this activation cannot be measured successfully because the action of the activated agent is on the platelets. In platelet-poor plasma, shortening of the clotting time is observed probably because platelets are not completely removed, and therefore, enough thrombin can be generated to bring about a normal clotting time, but insufficient prothrombin is consumed to be measurable. When platelet-rich plasma is clotted in a silicone-coated tube, the utilization of prothrombin also is so minute that it cannot be meas-

ured. In glass, on the contrary, the amount of prothrombin consumed is relatively high, as seen in Table 1. The integrity of the platelets is not a factor because freezing and thawing, which disrupt these cells, do not induce prothrombin consumption in silicone.

The addition of aged normal serum to platelet-free plasma does not bring about utilization of prothrombin because the platelet factor is not supplied by serum. When, however, serum is added to platelet-rich plasma, it will cause prothrombin consumption even in a silicone-coated tube. This suggests that serum contains the factor that is activated by glass and so can react with platelets. Evidence has accumulated showing that this factor is neither thromboplastinogen nor PTC, but a distinct agent to which such names as "contact factor" and "Hageman factor" have been applied. Perhaps, Seegers' platelet co-factor 1[39] may not be antihemophilic globulin or thromboplastinogen but actually the contact factor.

Inspection of the findings in Table 1 suggests the interesting possibility that the product of the interaction of the platelets with the activated plasma factor may be identical with the agent in the erythrocyte, which has been designated "erythrocytin." It is to be noted that hemolysate brings about good prothrombin consumption when added to platelet-poor plasma even if the clotting is carried out in a silicone-coated test tube. The purified product, erythrocytin, has been isolated from hemolysate (Hussey and Kaser[16]). From its properties and its nitrogen-phosphorus ratio, it appears to be a phospholipid. Like hemolysate it brings about good consumption when added to platelet-poor normal plasma. Erythrocytin enters the clotting reaction directly, whereas platelets must first react with a plasma factor that is inactive in circulating blood and requires activation.

Further information concerning the relation of erythrocytin to the clotting mechanism comes from the observation that when whole blood is mixed with an equal volume of its own platelet-poor plasma, the prothrombin consumption of the mixture is surprisingly poor though it is carried out in glass. This contrasts strikingly with the normal prothrombin consumption of undiluted whole blood or of a mixture of platelet-rich and -poor plasma.

The poor consumption of prothrombin of whole blood when mixed with an equal volume of its own platelet-poor plasma is not due to an insufficient number of platelets, since good consumption is obtained with much lower platelet counts but under different conditions. The results clearly point to the erythrocytes as being responsible for the depression of the consumption of prothrombin. One may postulate that these cells either inhibit the early stage of coagulation or remove the reaction product resulting from the interaction of platelets with the activated plasma factor. The latter explanation is the more probable because it accounts for the high concentration of erythrocytin in the red cells. When the platelet-poor plasma is exposed to glass surface before mixing it with whole blood, good consumption of prothrombin occurs, because presumably more erythrocytin is formed and the capacity of the erythrocytes to remove it is exceeded.

The release of erythrocytin from intact red cells is difficult to demonstrate experimentally even *in vitro* and may not even occur in normal circulating blood. There is evidence, however, that erythrocytin can be liberated during coagulation in glass as shown in Table 4, but this may be due to me-

chanical injury of the red cells. The ease with which erythrocytin can be liberated emphasizes not only the need for meticulous technique in carrying out the prothrombin consumption test but also the caution that must be exercised in interpreting the results. Thus, the normal prothrombin consumption time that may be obtained in mild hemophilia when the test is carried out on whole blood is readily explained. Such blood contains sufficient thromboplastinogen to bring the consumption within the normal range when a small amount of erythrocytin is liberated from the red cells.

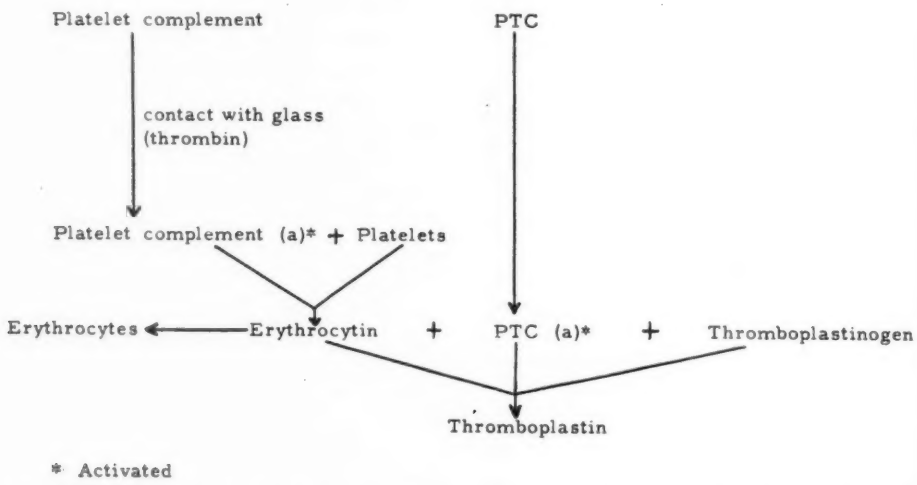

Fig. 2.—The relation of platelets to the clotting factor in erythrocytes (erythrocytin) in the production of intrinsic thromboplastin.

In a recent publication (Quick and Hussey[30]), it was postulated that one of the final steps in the formation of intrinsic thromboplastin is the interaction of erythrocytin, thromboplastinogen and activated PTC. On using the technique of the thromboplastin generation test, we have found that thromboplastin is produced by mixing purified thromboplastinogen, erythrocytin, and an eluate from aged normal serum. Prior to the final reaction in the production of thromboplastin, erythrocytin must become available and PTC requires activation. The first reaction in the clotting mechanism is that of platelets and a plasma factor which become activated by contact with the foreign surface such as glass. Since it has been shown that the addition of a minute quantity of thrombin brings about a good consumption of prothrombin when platelet-rich plasma is clotted in a silicone-coated test tube (Quick, Hussey and Epstein[31]), it seems likely that thrombin rather than the glass surface *per se* is responsible for activating the plasma factor. Such a surface adsorbs thrombin with avidity. It seems logical therefore that the thrombin film which perhaps is only a molecular layer is the true activator. It is to be emphasized that no surface similar to glass exists in the organisms. The agent which acts on the plasma factor is therefore more likely to be a compound than a catalyzing surface.

From the data presented, a tentative

scheme as given in Fig. 2 may be formulated to explain the formation of intrinsic thromboplastin. A factor in the platelet which is presumably a phospholipid type of compound interacts with a plasma constituent which is tentatively designated as "platelet complement." Perhaps, a further simplification could be achieved by calling the platelet agent, which some investigators designate as factor 3, "piastrin," from the Italian name "piastrine" for platelet. Before the platelet complement can react with the platelet factor, it must be activated. The product of the reaction is erythrocytin, which is in part taken up in the erythrocytes and in part enters into the production of intrinsic thromboplastin.

Since there is no evidence that intact red cells supply erythrocytin during coagulation, the amount that is removed from the blood by the red cells does not in all probability contribute to the generation of thromboplastin and in the utilization of prothrombin. Only the quantity produced in excess of that lost to the erythrocytes determines the amount of prothrombin consumption.

For erythrocytin to produce intrinsic thromboplastin, thromboplastinogen and PTC must be available. The latter component is in an inactive state in plasma but becomes activated during clotting. How this is brought about is not known but it is probably independent of the formation of erythrocytin.

The physiological purpose of erythrocytin in the red cell remains undetermined. It may represent a mechanism whereby this powerful clotting factor is promptly removed from the blood to prevent dangerous accumulation. Perhaps, too, it may function in the erythrocyte in some unsuspected manner that contributes to hemotasis. To be sure, this is mere speculation. Less hypothetical is the fact that when the erythrocytes undergo massive destruction in the bloodstream, a powerful clotting factor is liberated which can initiate coagulation similar to that observed when hemolysate is added to blood in a silicone-coated tube. It should again be emphasized that erythrocytin can promptly enter into the clotting reaction, for unlike platelets, it does not require preliminary activation. Perhaps there was a prophetic note in the statement of Wooldridge when, in 1886, he said: "The fact that the stromata cause intravascular clotting is in many ways interesting; and I trust will lead to important conclusions regarding the causation of thrombosis during life."

Summary and Conclusions. In a test tube, the initial reaction in the coagulation of blood is the activation of a plasma constituent by contact with the glass surface, whereupon it reacts with a platelet factor to produce an agent that appears to be identical in its activity to the clotting factor, erythrocytin, which is present in the erythrocyte.

Evidence that the erythrocytin is formed in the plasma and taken into the red cell is obtained from the observation that the consumption of prothrombin is poor when whole blood is mixed with an equal volume of its own platelet-poor plasma that has not been in contact with glass prior to mixing.

The consumption of prothrombin in the clotting of normal blood is dependent in part on the amount of erythrocytin produced in excess of the quantity taken into the erythrocyte.

The erythrocytin in intact erythrocytes is apparently not readily available for coagulation but slight injury of the red cell can cause its liberation. The effect of this on the prothrombin consumption time is discussed and the probable effect of massive intravascular hemolysis is pointed out.

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(Interlingua on page 10)

THE INTRAVENOUS CONGO RED TEST: A DESCRIPTION OF SOME UNRECOGNIZED FALLACIES WITH SUGGESTED IMPROVEMENTS*

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Stimulated by the statement that some patients with rheumatoid arthritis in the absence of amyloidosis show abnormal Congo Red retention (Dixon, Ramcharan and Ropes[3]) and by the failure of other observers to notice this abnormality in a similar series (Unger *et al.*[10]), we began a series of intravenous Congo Red tests in arthritic patients. It soon became evident that accepted methods of performing the test were faulty and it was thought desirable to study the test in detail. We concluded from our studies that much could be done to improve its accuracy and that comparison of results from different sources is not possible unless the test has been performed by the same method. In published reports we have noticed that the method is only occasionally described and that more frequently the percentage absorption of the dye is given with no further reference to the method used. Such figures are of limited value in the absence of unequivocal amyloidosis.

Materials and Methods. The test was performed in over 40 patients, the majority of whom were attending an arthritis clinic. Of these, 23 had definite and in many instances severe rheumatoid arthritis, a few had other arthritides, some of which were probably early atypical rheumatoid and a further small group had general medical diseases. As some of the fallacies in the test became apparent, the samples of serum obtained from a patient undergoing the test were treated by both the original and the modified methods. On 3 occasions the complete test was repeated. At the commencement of the series we adopted the same method for the test as Unger *et al.*[10] with certain minor differences.

Preparation of a standard solution. One ml. of 1% aqueous Congo Red is diluted to 500 ml. with distilled water. From this stock solution 10, 25, 37.5, 50 and 75% solutions are prepared which are then read colorimetrically. A 1:500 dilution of 1% aqueous Congo Red in a 1:1 acetone-water mixture is also prepared and dilutions similar to those made with distilled water are prepared and read colorimetrically. The readings obtained with a Klett-Summerson Photoelectric Colorimeter at a wave length of 540 are transformed into percentage transmission and plotted against concentration on semi-logarithmic paper.

The acetone-water and the distilled water standards gave readings that differed considerably. The readings obtained with the water standard were reproducible while those of the 1:1 mixture were not; it was, therefore, decided to use only the water standard in calculating the absorption results.

Technique. The determination is made on fasting subjects in order to avoid lipemia.

A 10 ml. specimen of blood is removed prior to injection; this serves as the blank control. One milliliter of 1% aqueous Congo Red per 10 lbs. of body weight is then injected, a few ml. of blood are then withdrawn into the syringe and re-injected so that all the dye is washed out of the needle and nozzle. From the opposite arm, 10 ml. blood samples are removed at 2, 4, 6, 8, 10, 20, 30, 45 and 60 minutes after injection.

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Where possible the original needle is left in place for the withdrawal of the first 5 specimens, no anticoagulant being required.

Where there were good veins available in only one arm, the blank sample was removed and the dye injected into the external jugular vein on the opposite side from the arm intended to be used for removal of the timed specimens.

The blood is allowed to stand for 1 hour at room temperature and then for 30 minutes in the refrigerator. The serum is separated by centrifugation and 2 ml. aliquots of each test sample are placed in centrifuge tubes.

To each of these tubes is added 6 ml. of acetone. The tubes are stoppered and shaken to ensure mixing and precipitation of the hemoglobin and protein. The specimens are then centrifuged for 30 minutes at 3,000 r.p.m.

A crystal clear solution of Congo Red is usually obtained but this solution often becomes cloudy on standing and centrifugation does not always remove the cloudiness. If after a second centrifugation the solution is clear, it too can become cloudy rapidly.

The supernatant is decanted into colorimeter tubes and read in the colorimeter at a wave length of 540.

The untreated serum does not lose an appreciable amount of color intensity after standing in the refrigerator for several days.

CALCULATIONS OF RESULTS. The concentration is expressed in ml. of 1% Congo Red per liter of serum. The transmission values of the various specimens are converted into concentration from the standard curve. These values are then multiplied by the dilution used in preparing the protein-free filtrates, in this case 4. A graph is constructed by plotting time against concentration of the dye on semilogarithmic paper and the zero concentration derived by extrapolation. Further detailed reference to this will be made later.

The intravenous Congo Red Test was introduced by Bennhold[1] as a test for amyloidosis. Since its original description various modifications have been suggested to improve its accuracy. As the test was first described, an intravenous injection of 10 ml. of 1% aqueous Congo Red was given in the fasting state. It had been shown that lipemia interfered with the color of the serum, so specimens were taken after a 10-hour fast. At 4 and 60 minutes after the intravenous injection, specimens of blood were removed and the percentage transmission of the serum was compared colorimetrically. The 4-minute specimen was regarded as containing an amount of Congo Red equivalent to a specimen removed at zero time. Later it was shown that turbidity or slight hemolysis of the serum could be responsible for inaccuracies in the test (Friedman and Auerbach[4] and Taran[8]). Friedman suggested adding to the specimen 5 times the volume of ethyl alcohol and thus precipitating any hemoglobin liberated by hemolysis. The degree of dilution resulting from this procedure was sufficient to cause errors in the colorimetric readings as the specimens did not always contain sufficient dye. Taran[8] therefore recommended the addition of an equal volume of acetone which he claimed precipitated any hemoglobin, cleared any turbidity of the serum and enabled more accurate readings to be obtained. Taran and Eckstein[9] found that a 4-minute specimen was frequently too light to be relied on as the standard, because in cases of amyloidosis uptake of the dye can be very rapid. They recommended that a 2-minute specimen be taken as well and that it should be regarded as the standard. In order to obtain comparable readings in the 2- and 4-minute specimens irrespective of the size and weight of a patient, they suggested giving 1 ml. of the dye per 10 lbs. of body weight. Harmon and Kernwein[6] introduced a further modification by using known standard solutions of Congo Red for comparison, thereby allowing the test to be put on a quantitative basis.

Further improvements were suggested by Unger *et al.*[10]. They pointed out that the use of a 2- or 4-minute specimen to represent the concentration at zero time was invalid. Complete mixing of the dye does not take place for 7 to 10 minutes and may take longer in patients with a slowed circulation time (Gibson and Evans[5]). On the other hand, the assumption that no absorption of Congo Red takes place in the first 4 minutes in a person with amyloidosis is also unwarranted. Unger *et al.*[10] felt that it was desirable to derive a figure which would represent the concentration of the dye at zero time. The method they recommended was to take specimens at 2, 4, 6, 8, 10, 20, 30, 45 and 60 minutes instead of only at 2, 4 and 60 minutes. They were able to draw a disappearance curve for amyloid and for normal subjects, Fig. 1. In a normal or non-amyloid person, if these values were plotted on semi-logarithmic paper, a straight line was obtained when points between 10 and 60 minutes were used, Fig. 2. Earlier points are not suitable because mixing is incomplete, but extrapolation of this line enabled the zero concentration to be derived.

In amyloidosis, connection of the points in

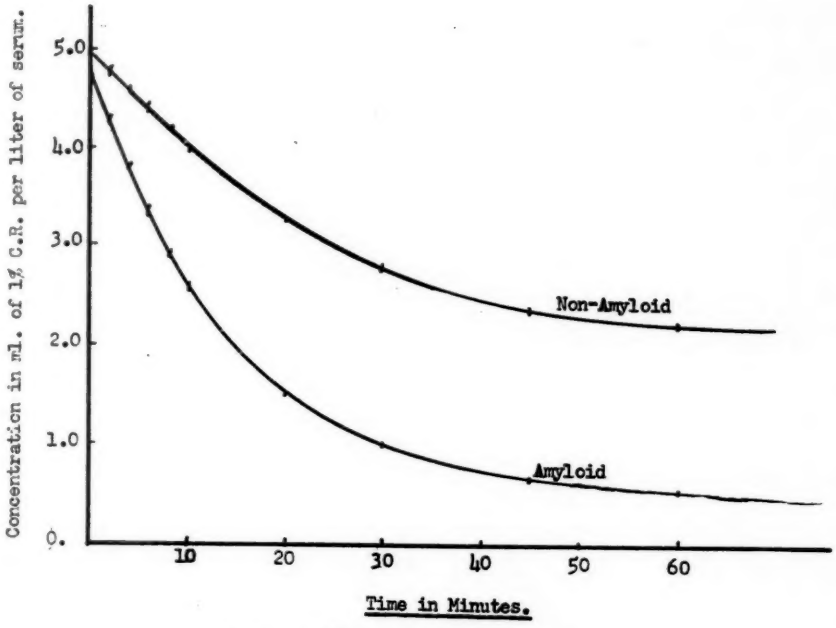

Fig. 1.—Disappearance curve of C.R.

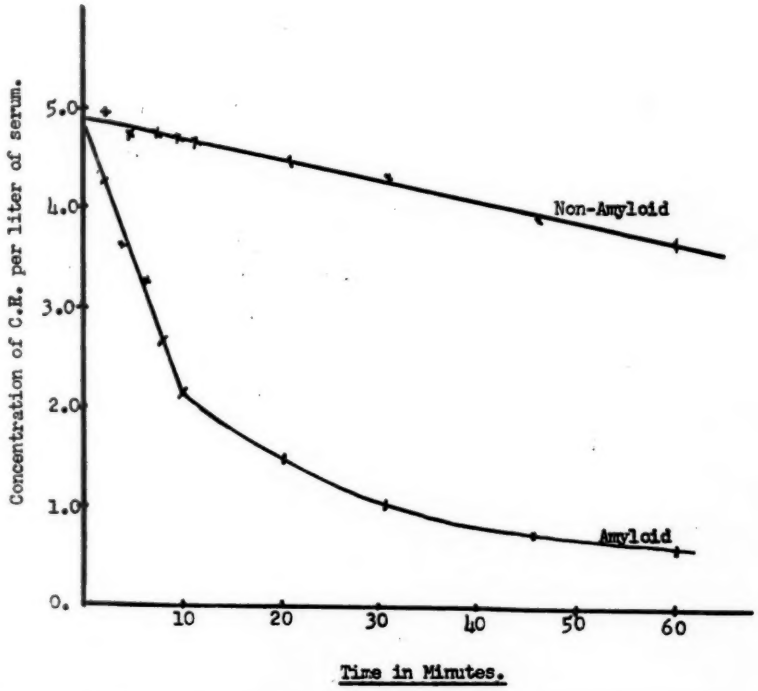

Fig. 2.—Disappearance curve of C.R. plotted on semi-logarithmic paper.

the graph plotted on semi-logarithmic paper produced not a straight line but a curve. It was felt that extrapolation of the line connecting the early specimens (2 to 10 minutes) would give the most accurate value for the zero concentration of the dye, Fig. 2.

Using curves plotted in this fashion, it was shown that when amyloid was present, the dye absorption was virtually complete within the first 30 minutes. In the same paper the authors derived the concentration at zero time by simple calculation knowing the amount of Congo Red injected and obtaining the plasma volume from standard tables based on height, weight, and surface area.

In essence the Congo Red test is based on the fact that amyloid tissue removes the dye from serum. An absorption of 60% or more in one hour is strongly suggestive of amyloidosis. Some dye is taken up by the reticuloendothelial system and a negligible quantity is excreted in the urine (Unger *et al.*[10]). Inaccurate results could arise in the performance and technique of the test. A further source of unexpected or incongruous results may be attributable to the following assumptions, neither of which is probably correct.

A. That the dye is completely free to pass to amyloid tissue in the body. It may be that the protein constituents of the serum and amyloid tissue have to compete for the dye. Larsen[7] has produced *in vitro* evidence that the binding capacity of the serum for the dye depended on the level of serum albumin and varied inversely with the concentration of the serum glycoproteins.

B. That all forms of secondary amyloid take up the dye with equal avidity. It is known that the amyloid found in primary amyloidosis does not take up the dye so rapidly but it is often assumed that all forms of secondary amyloidosis do so with the same avidity.

As has been mentioned, when standard solutions of the dye in water and in a 1:1 acetone and water mixture were prepared, the 2 solutions were found to have different percentage transmissions and furthermore the colors of the solutions differed even to the naked eye. This had not been noted in previous publications and led us to consider the technique of the test in detail.

When sufficient serum remained from a patient having the test, a second series of readings was obtained from the specimens. It was the custom to dilute 2 ml. portions of serum with 6 ml. of acetone but when it was possible to repeat the procedure, the 2 ml. of serum was diluted with only 2 ml. of acetone. Although the serum used in each instance contained the same amount of dye, the concentrations obtained when the specimens were diluted 1:1 were always significantly lower. Unger *et al.*[10] state precipitation with acetone does not remove any dye from the serum. Our experience differs. Several experiments were devised to show that this statement is incorrect. Table 1 shows the concentrations of dye obtained by diluting with acetone serum containing the same amount of Congo Red by 1:1 and by 1:3. It will be seen that the 1:3 dilutions gave considerably higher results.

Table 2 shows a recovery experiment in which 1-ml. portions of serum containing a known amount of Congo Red were treated increasing volumes of acetone; thus to the first specimen of serum, 1 ml. of acetone was added, to the second, 2 ml. of acetone and so on up to 6 ml. of acetone. The concentrations were then read from the standard graph and it will be seen that they are the same with the weaker dilutions but that the 1:1 and 1:2 dilutions differ markedly. It was concluded, therefore, that some dye was lost in the protein precipitate and this was borne out by

inspecting the color of the precipitate which to the naked eye was obviously much more intense in the 1:1 dilution than in any of the other precipitates.

To assume that the concentration of the dye at zero time can be calculated from the volume of the dye injected and the plasma volume which is derived from standard tables is often unwarranted. In many instances a reasoning the degree of dilution of an intravenous dose of iodinated human serum albumin tagged with I_{131} (IHSA). Estimations of plasma volume using this principle are well described by Berson and Yalow[2] and are more easily performed than those methods using Evans blue. To illustrate the disparity that exists between values obtained from standard tables and those actually

TABLE 1.—CONCENTRATION OF CONGO RED OBTAINED IN SAME SPECIMENS OF SERUM TREATED WITH DIFFERENT VOLUMES OF ACETONE.

Time in minutes.	2	4	6	8	10	20	30	45	60
Concentration in ml. of 1% C.R. per liter as obtained by treatment with a 1:1 dilution of acetone.	4.00	4.10	4.12	4.10	—	3.88	3.70	3.52	3.37
Concentration in ml. of 1% C.R. per liter obtained by treatment with a 1:3 dilution of acetone.	6.40	6.40	6.16	6.52	7.40	5.52	5.87	5.40	5.40

TABLE 2.—TO DEMONSTRATE LOSS OF CONGO RED WHEN SERUM IS TREATED WITH DIFFERING VOLUMES OF ACETONE.

Volume of serum containing known and same amount of C.R.	*Treated with increasing volume of acetone.*	*Actual concentration* in ml. of C.R. found after treatment.*
1 ml.	1 ml.	3.80
1 ml.	2 ml.	4.50
1 ml.	3 ml.	4.48
1 ml.	4 ml.	4.60
1 ml.	5 ml.	4.50
1 ml.	6 ml.	4.48

*After volume had been adjusted to a total of 7 ml.

ably accurate estimate can be arrived at but in rheumatoid arthritis there is often an appreciable increase in the plasma volume. This has been observed by several authors including Dixon, Ramcharan and Ropes[3]. Our own investigations have confirmed the increased plasma volume in this condition which, although usually moderate, can be marked. The method of determination used by us depended on measurmeasured by IHSA, it is appropriate to consider one case of Still's disease who had an estimated volume of 1960 ml. but a measured volume of 3100 ml. Steroids further modify both the red cell mass and the plasma volume. As a result of these determinations using IHSA, we concluded that in rheumatoid arthritis the figures given for plasma volumes in standard tables are often erroneous. Table 3 compares the

2 methods of arriving at a figure for the plasma volume in a series of 12 patients.

In an attempt to assess the accuracy of the concentration at zero time derived by extrapolation, we decided to compare this with the concentrations calculated from standard tables and by IHSA determinations, the latter method being regarded as the most accurate. These results are illustrated in Table 4. It is seen that in 7 out of 11 cases the concentration derived by extrapolation is significantly higher than the concentration derived from standard tables and in 8 out of 11 is significantly higher than the concentration derived by IHSA measurements. Comparison of the values obtained from tables and by IHSA measurements shows that in

TABLE 3.—TO SHOW PLASMA VOLUMES FROM STANDARD TABLES AND THOSE CALCULATED WITH I.H.S.A.

Patient	*Sex*	*Diagnosis*	*P.V. from standard tables*	*P.V. from I.H.S.A.*
C.M.	M	Still's Disease.	1960 ml.	3100 ml.
G.S.	M	R.A.	2640 ml.	2850 ml.
E.R.	F	R.A.	2060 ml.	2440 ml.
L.B.	M	Cirrhosis of liver.	3660 ml.	4860 ml.
A.D.	F	R.A.	2440 ml.	3126 ml.
S.B.	F	R.A.	2560 ml.	2700 ml.
R.C.	F	Asymmetrical polyarthritis probably R.A.	2560 ml.	2420 ml.
A.B.	F	R.A.	2690 ml.	2630 ml.
J.D.	M	Pachyderma Periostitis & R.A.	2640 ml.	3120 ml.
K.C.	F	R.A.	1930 ml.	2010 ml.
L.G.	M	R.A.	2800 ml.	3550 ml.
L.D.	F	Sarcoidosis.	2480 ml.	2950 ml.

(R.A. = Rheumatoid Arthritis)
(P.V. = Plasma Volume)

TABLE 4.—TO SHOW ZERO CONCENTRATIONS OBTAINED BY EXTRAPOLATION, BY USING STANDARD TABLES AND BY ACTUAL DETERMINATION OF PLASMA COLUMN WITH I.H.S.A.

Patient	*Sex*	*Diagnosis*	*Zero concentration from extrapolation*	*Zero concentration from standard tables*	*Zero concentration from I.H.S.A.*
C.M.	M	Still's Disease.	3.90	4.72	3.23
G.S.	M	R.A.	6.00	5.19	5.26
E.R.	F	R.A.	6.40	5.26	4.50
A.D.	M	R.A.	5.20	5.02	4.31
S.B.	F	R.A.	5.80	5.19	5.55
R.C.	F	Asymmetrical poly-arthritis probably R.A.	5.75	5.00	5.36
A.B.	F	R.A.	6.15	4.15	4.16
J.D.	M	Pachyderma Periositits & R.A.	5.80	4.84	4.49
R.C.	F	R.A.	4.75	5.02	4.98
L.G.	M	R.A.	6.75	5.16	4.51
L.D.	F	Sarcoidosis.	5.55	5.20	4.75

Each value is expressed in ml. of 1% C.R. per liter of serum.

5 out of 11 cases there is a reasonable relationship between the zero concentrations, but in 6 out of 11 cases there is a moderate to considerable divergence, this being attributable to the dye being diluted more than was expected.

As described, because of the disparity in readings between the standard solutions made with distilled water and with 1:1 acetone water mixture, we decided to use the former standard as this gave us consistent readings. As the zero concentration derived from extrapolation was consistently too high, that is to say more dye was being found in the serum than was being injected, it was concluded that the standard solution of dye in distilled water was giving readings which were too high. This again shows the fallacy of applyin *in vitro* findings to real life. To simulate more closely *in vivo* conditions, a standard solution was made with serum.

PREPARATION OF A STANDARD SOLUTION USING SERUM. Since it was decided that the comparison of the test specimens to a water standard gave falsely high results because of the difference in behavior of the dye itself in water and in serum, a standard composed of 1 ml. of 1% Congo red in 250 ml. of pooled normal serum was prepared. From this stock solution, dilutions are made in the following manner:—

Serum	*Stock solutions of serum and C.R.*	*Dilution*
2.0 ml.	—	0%
1.5 ml.	0.5 ml.	25%
1.0 ml.	1.0 ml.	50%
0.5 ml.	1.5 ml.	75%
—	2.0 ml.	100%

Duplicate and triplicate dilutions are made in this way. Each of the specimens of each dilution is then treated with acetone as if it were a specimen containing an unknown quantity of the dye, that is, to each specimen of 2 ml. is added 6 ml. of acetone. This simulates as closely as possible *in vivo* conditions. The percentage transmission of each sample is then determined and is plotted on semilogarithmic paper.

The standard solution made up in this fashion had very different physicochemical properties from that made with distilled water. Different percentage transmissions were found for the 2 standard solutions and their respective dilutions. Table 5 shows the zero concentrations derived by extrapolation using the aqueous standard, as compared with the concentrations derived by using the serum standard and that determined with IHSA. In every instance the concentration in column II is lower than in column I. The values in column II also bear a better relationship to those derived by IHSA measurements in column III. In 5 out of 11 cases the values do not differ significantly. The disparities between columns II and III still caused concern although we felt some of the difference may be accounted for by the fact that there was an interval of 2 to 3 days between the Congo red test and the measurement of the plasma volume.

It is felt that the modifications suggested increase the accuracy of the test but still leave something to be desired. Most of the difficulties arise with the use of acetone. It is thought that some form of treatment of the serum is desirable to remove any hemoglobin, to clear any turbidity and also as the values obtained by reading untreated serum are either off the colorimeter scale or on the most inaccurate part of it. Acetone has the following disadvantages:—

1. It precipitates some of the dye along with the protein when it is added to serum in equal proportions.
2. It evaporates readily and is difficult to pipette.
3. It does not always clear the serum of cloudiness.

4. It is an organic solvent and it is theoretically preferable not to treat serum containing a water soluble dye with it.
5. Acetone and water form mixtures which have different physico-chemical properties depending on the proportion of the constituents.

It was therefore decided to try and find an agent which would not have the disadvantages of acetone. This proved quite difficult but after trials of several agents, sodium sulfate was tried and it is felt that this agent will prove more satisfactory than acetone. A new procedure was devised and details of it are given below.

METHOD USING SODIUM SULFATE. As with acetone, a stock solution made with the dye and sodium sulfate gave results which were erroneously high. A standard solution was made using pooled normal serum as in the modified acetone method. Each specimen of each solution is then treated with 4 ml. of sodium sulfate solution containing 23 gm. per liter. This means that 2 ml. portions of the serum containing the dye are treated with 4 ml. of sulfate solution, requiring that each percentage transmission obtained be multiplied by 3 rather than 4, as in the method using acetone. The specimens were read at a wave length of 500, this having been determined as the most satisfactory. The values obtained were then plotted on semi-logarithmic paper as before. It was found that the stock solution of dye in serum is not as stable as that made with the dye and water only. The graph is therefore constructed on the same day as the standard solution is made up.

Every specimen which has been used in the plotting of the graph has been treated with sulfate so it is not possible to know whether any of the dye has been precipitated and accordingly it is thus impossible to devise a recovery experiment in which the amount of dye in a specimen is estimated with and without sulfate precipitation. The precipitate formed by the addition of sulfate is white except where the supernatant is in intimate contact and when the latter is decanted, the addition of a little more sulfate leaves a pure white precipitate after further centrifugation. This suggests that no dye is bound to the precipitate. Table 6 shows an experiment where 1-ml. por-

TABLE 5.—TO SHOW ZERO CONCENTRATIONS OBTAINED BY EXTRAPOLATION USING A STANDARD SOLUTION OF THE DYE IN WATER (COLUMN I), USING A STANDARD SOLUTION MADE WITH SERUM (COLUMN II), AND THAT DETERMINED WITH I.H.S.A. (COLUMN III).

Patient	*Sex*	*Diagnosis*	I *Extrapolated value*	II *Extrapolated value*	III *I.H.S.A. value*
C.M.*	M	Still's Disease.	3.90	3.20	3.23
G.S.	M	R.A.	6.00	5.00	5.26
E.R.	F	R.A.	6.40	5.20	4.50
A.D.	M	R.A.	5.20	4.25	4.31
S.B.	F	R.A.	5.80	4.80	5.55
R.C.	F	Asymmetrical poly-arthritis probably R.A.	5.75	4.75	5.36
A.B.	F	R.A.	6.50	5.05	4.16
J.D.	M	Pachyderma Periostitis & R.A.	5.80	4.80	4.49
K.C.	F	R.A.	4.75	3.85	4.98
L.G.	M	R.A.	6.75	5.70	4.51
L.D.	F	Sarcoidosis.	5.55	4.70	4.75

Each value is expressed in ml. of 1% C.R. per liter of serum.

tions of serum containing the same amount of dye were treated with increasing volumes of sulfate and then each specimen was made up to 7 ml. with distilled water. The colorimeter reading of each specimen was essentially similar. This is in contrast to a similar procedure in which acetone was used (Table 2). A further test of the first 2 cases is probably due to the plasma volume being measured a few days after the Congo red test but even so the results do not differ by more than 10%.

Discussion. It is thought that our results with the test in over 40 cases may be of interest. It must be stressed that the percentage tissue absorption of Congo red is usually expressed simply as a figure; this means very little unless the method of performance of the test is stated. Figures differing by as much as 30 to 40% can be arrived at in the same test depending on the method of calculation and the manner in which the serum containing the dye is treated. This is especially evident when the zero concentration is calculated from standard tables of plasma volume. Most of the cases in this series came from an arthritis clinic. In only the supposition that no significant quantity of dye is lost by precipitation would be to compare the zero concentration found by extrapolation with that measured by means of IHSA. Table 7 illustrates the close proximity of the 2 sets of figures and also compares them to the zero concentration derived by the modified acetone method. As can be seen all 3 methods give closely related values, but for the reasons already listed the sulfate method is preferred. The rather larger difference in

TABLE 6.—1 ML. PORTIONS OF SERUM CONTAINING SAME AMOUNT OF C.R. TO WHICH ARE ADDED INCREASING VOLUMES OF SODIUM SULFATE AND THEN DISTILLED WATER TO INCREASE TOTAL VOLUME TO 7 ML.

Volume of serum containing C.R.	*Volume of sodium sulfate.*	*Volume of water added.*	*Colorimeter reading.*
1 ml.	1 ml.	5 ml.	236
1 ml.	2 ml.	4 ml.	236
1 ml.	3 ml.	3 ml.	236
1 ml.	4 ml.	2 ml.	238
1 ml.	5 ml.	1 ml.	240
1 ml.	6 ml.	—	236

TABLE 7.—COMPARISON OF ZERO CONCENTRATIONS DERIVED BY EXTRAPOLATION SERUM STANDARD (I), SULFATE AND SERUM STANDARD (II) AND BY ACTUAL I.H.S.A. DETERMINATION (III).

Patient	*Sex*	*Diagnosis*	I *Zero concentration acetone*	II *Zero concentration sulfate*	III *I.S.H.A. value*
G.W.	M	R.A.	3.95	4.05	3.65
V.P.	M	Osteoarthritis.	4.90	5.20	4.75
L.D.	F	Sarcoidosis.	4.70	4.85	4.75
H.C.	M	Cerebral thrombosis.	4.50	4.62	4.35
W.J.	M	Hepatic Cirrhosis.	3.25	3.10	3.22

Each value is expressed in ml. of C.R. per liter of serum.

3 out of 23 cases of rheumatoid arthritis were values of greater than 30% found when a serum standard was used and the zero concentration derived by extrapolation. The tissue absorption in these cases was only increased to between 35 and 45% in 1 hour although much higher values could be derived if the zero concentration was obtained from standard tables of plasma volume. Many patients had severe long-standing arthritis in whom amyloidosis may reasonably have been suspected. Our findings, therefore, are not in agreement with those of Dixon, Ramcharan and Ropes[3]. Absorptions of significantly over 30% were found in 3 other patients, one with serum sickness, one with an arthralgia probably due to brucellosis and one with hepatic cirrhosis. Two cases of Reiter's syndrome, one case of severe arthropathic psoriasis and one of osteitis condensans ilii had normal values. It is hoped to extend the study to a larger group of patients with rheumatoid arthritis in order to try and substantiate the findings of Dixon, Ramcharan and Ropes[3]. If an increased tissue uptake of Congo red can be demonstrated it is clearly desirable to determine the cause which may be of considerable importance.

Summary. 1. The accuracy of the intravenous Congo red test was investigated. Reasons are given for the inaccuracies revealed.

2. Modifications are suggested to overcome these deficiencies. The importance of preparing standard solutions with serum is stressed as are the disadvantages of using acetone as an agent for treating the serum.

3. A study was made of the test in a group of arthritic and other patients. The results show that a few cases of rheumatoid arthritis have an increased tissue absorption of the dye. Whether the phenomenon is more frequent than in other diseases is open to doubt.

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(Interlingua on page 26)

THE EFFECT OF DESICCATED THYROID ON THE HYPERLIPOPROTEINEMIA OF XANTHOMA TENDINOSUM*

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Xanthoma tendinosum is a disease characterized by elevated blood lipids (Gofman *et al.*[4], Guravich[5]), particularly the $s_f^0$0–20 serum lipoproteins, and therefore, also, serum cholesterol. An hereditary factor is involved since it has been recognized that xanthoma tendinosum occurs in families (Guravich[5], Harris-Jones, Jones and Wells[6], Leonard[7], Piper and Orrild[8], Wheeler[16]). Treatment of the blood lipid disorders characteristic of this disease has centered about dietary measures designed to lower serum lipids, especially cholesterol (Urbach, Hildreth and Wackerman[15]).

In intensive studies of the hyperlipoproteinemia associated with xanthoma tendinosum, Gofman found, in a group of 18 patients, that the low density serum lipoproteins $s_f^0$0–12, $s_f^0$12–20, and $s_f^0$20–100 were significantly elevated and $s_f^0$100–400 significantly decreased in concentration compared with the lipoprotein values observed in a series of controls matched by age and sex (Gofman *et al.*[4]). The high density lipoprotein HDL-1 was significantly elevated in concentration compared with the matched control group, and the HDL-2 and HDL-3 high density lipoproteins were significantly decreased.

A study of the hyperlipoproteinemia associated with spontaneous myxedema (Gofman *et al.*[4]) revealed a striking similarity of the lipoprotein derangements of xanthoma tendinosum and spontaneous myxedema, as shown graphically in Fig. 1. Clinical examination of all of the xanthoma tendinosum patients in this series failed to reveal any evidence of thyroid dysfunction. The protein bound iodine measurements done were within the euthyroid range.

Coupled with this unique and striking similarity of serum lipoprotein concentrations in xanthoma tendinosum and spontaneous myxedema was the knowledge that desiccated thyroid exerts a powerful and specific hypolipoproteinemic effect on the $s_f^0$0–12 and $s_f^0$12–20 serum lipoproteins in euthyroid individuals (Strisower *et al.*[10,11,12]). Furthermore, the higher the initial $s_f^0$0–12 and $s_f^0$12–20 lipoprotein concentrations, the greater the hypo-

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lipoproteinemic effect of desiccated thyroid observed (Strisower *et al.*[10,12]).

It is our purpose to describe a long-term study of the use of desiccated thyroid in the control of the hyperlipoproteinemia associated with xanthoma tendinosum.

The group of 20 patients studied include 12 individuals with the lesions of xanthoma tendinosum and the associated typical lipoprotein findings, and under study. The age range was 5 to 68 years. Thirteen females and 7 males were studied for an average of 125 weeks, or over 2 years (range 38 to 201 weeks).

Administration of desiccated thyroid was begun with a dose of 1 grain per day and increased by 1 grain per day at approximately one-month intervals until a maintained hypolipoproteinemic response was achieved. The maximum

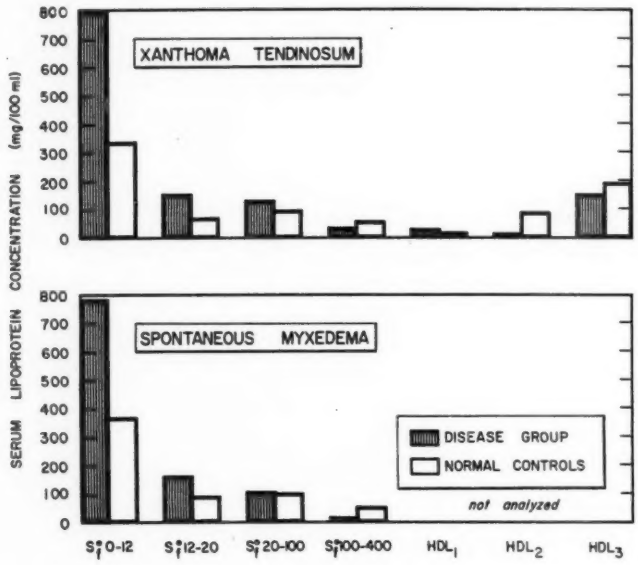

Fig. 1.—The bar graphs compare the disease groups xanthoma tendinosum and spontaneous myxedema with normal control groups matched by age and sex with the respective disease groups.

8 individuals (related by blood with a patient with xanthoma tendinosum in this series) with the typical lipoprotein distributions of xanthoma tendinosum *without* the tendinous lesions. In only one of the patients, ME, was there any evidence of clinical coronary heart disease; in this case, questionable angina pectoris.

In Table 1 are given the age and sex of each patient, diagnosis, relationship with other members of the group, and total length of time each patient was dosage of desiccated thyroid, which averaged 6 grains per day (range 3 to 10 grains per day), was continued for an average of 36 weeks (range 9 to 104 weeks).

From all patients 2 to 4 initial pretherapy blood samples were drawn. Blood samples were drawn during the on-therapy period at 1- to 3-month intervals. All blood samples were analysed ultracentrifugally for low and high density serum lipoproteins by the method of deLalla and Gofman[2]. Total

serum cholesterol analyses were performed on all blood samples by the method of Colman[1]. Body weight was recorded at the time of blood sampling.

Initial pre-therapy sample results refer to the mean of two to four samples drawn before thyroid administration was begun. On-therapy results are based on the mean of several samples drawn during the periods on the *maximum* dosage of desiccated thyroid achieved by each patient. Table 2 gives the detailed low and high density lipoprotein and cholesterol data for the xanthoma tendinosum group The pre-therapy means and ranges are given, as well as the on-therapy changes for the 6 adult males, 10 adult females and 4 children comprising the total group. Because the variations in the results of the three groups are small, the results were combined for statistical analysis.

TABLE 1.—FAMILY RELATIONSHIP AND CLINICAL INFORMATION IN XANTHOMA TENDINOSUM GROUP

Patient	*Age/Sex*	*Diagnosis**	*Relationship*	*Total Time Treated (wks)*
DAc	48M	Xanth. Tend.	——	113
DA	5M	No lesions	2nd cousin of JC, Xanth. Tend.	146
MA	30F	No lesions	Mother of DA, 1st cousin of JC, Xanth. Tend.	148
RA	40F	No lesions	Sister of† Xanth. Tend.	105
BB	10F	No lesions	Daughter of PB, Xanth. Tend.	181
PB	33F	Xanth. Tend.	Mother of BB	201
AC	8F	No lesions	Daughter of WC, Xanth. Tend.	134
WC	40M	Xanth. Tend.	Father of AC	128
JC	41F	Xanth. Tend.	1st cousin of MA 2nd cousin of DA	126
ME	51F	Xanth. Tend. and Tub.	——	193
RF	22F	No lesions	Sister of RT, Daughter of Xanth. Tend.	137
CG	5F	No lesions	Niece of PB, Xanth. Tend., Daughter of Xanth. Tend.	152
LG	17M	Xanth. Tend.	——	88
JH	49M	Xanth. Tend.	——	38
GL	58F	Xanth. Tend.	Sister of PS Xanth. Tend.	102
HM	22F	Xanth. Tend.	Daughter of JC Xanth. Tend.	139
DS	37M	Xanth. Tend.	Son of PS Xanth. Tend.	75
PS	68F	Xanth. Tend.	Mother of DS Sister of GL Xanth. Tend.	101
RT	30F	No lesions	Daughter of Xanth. Tend, Sister of RF	148
WW	49M	Xanth. Tend.	——	54

*All these patients had the typical lipoprotein findings of xanthoma tendinosum with or without the tendinous lesions.

†Relationship: If no initials are given the relationship is to a relative whom we had studied and who had died. For example RT and RF are sisters. Their mother EN, a xanthoma tendinosum, had died before this study was begun.

TABLE 2.—LIPOPROTEIN FINDINGS IN EXANTHOMA TENDINOSUM GROUP

		Adult Males Xanth. Tend. Families	*Adult Females Xanth. Tend. Families*	*Children Xanth. Tend. Families*	*Summary Xanth. Tend. Families*
N		6	10	4	20
Age	Mean	40	40		35
Years	Range	17–49	22–68	5–10	5–68
Max Dosage	Mean	6	7	5	6
Grains	Range	3–10	3.5–10	4–6	3–10
Duration	Mean	24	31	68	36
Weeks	Range	12–36	9–62	13–104	9–104
Weight	Initial*	179	135	60	
Lbs.	Range†	156–195	98–166	41–85	41–195
	Change	−15	−6	+7	
s_f^0*–12*	Initial	873	835	752	829
mg /100ml.	Range	553–1273	533–1095	642–844	533–1273
	Change	−175	−228	−281	−223
	Sign.				p<0.001
s_f^{12}*–20*	Initial	153	135	99	133
mg./100ml.	Range	126–201	61–196	51–131	51–201
	Change	−61	−45	−49	−51
	Sign.				p<0.001
s_f^{20}*–100*	Initial	139	94	52	99
mg./100ml.	Range	75–242	28–248	41–66	28–248
	Change	−8	−4	−6	−6
	Sign.				NS
s_f^{100}*–400*	Initial	37	15	6	20
mg./100ml.	Range	4–86	0–79	0–10	0–86
	Change	+13	+11	+6	+10
	Sign.				NS
A.I.	Initial	145	126	103	127
Units	Range	113–164	73–176	81–117	73–176
	Change	−27	−29	−37	−30
	Sign.				p<0.001
Cholesterol	Initial	501	455	415	461
mg./100ml.	Range	363–696	314–545	364–452	314–696
	Change	−128	−113	−142	−123
	Sign.				p<0.001
HDL-1	Initial	29	25	35	28
mg./100ml.	Range	24–39	17–35	26–43	17–43
	Change	−3	−6	−12	−6
	Sign.				p<0.01
HDL-2	Initial	30	44	46	40
mg./100ml.	Range	10–62	11–90	33–60	10–90
	Change	+14	0	+5	+6
	Sign.				NS
HDL-3	Initial	194	209	196	201
mg./100ml.	Range	161–224	124–253	148–249	124–253
	Change	+8	+2	+7	+5
	Sign.				NS

*Initial mean value
†Range of initial values

Both the s_f^o0–12 and s_f^o12–20 low density lipoprotein concentrations were significantly reduced by the administration of desiccated thyroid, but no changes in the s_f^o20–100 or s_f^o100–400 low density lipoprotein concentrations were observed. The Atherogenic Index (AI)* and the serum cholesterol concentration were significantly reduced by the thyroid administration. The only significant change in the high density lipoproteins observed was the drop in HDL-1 concentration. HDL-1 is the only high density lipoprotein which is significantly elevated in xanthoma tendinosum†.

One patient, ME, had extensive flat yellow-orange lesions covering the palm of each hand when first studied; these lesions completely disappeared with the administration of desiccated thyroid and the resultant lowering of the s_f^o0–20 serum lipoprotein concentrations in this case. No change was observed in the size, texture, or color of the tendinous lesions or the xanthelasma in those patients exhibiting these lesions.

The s_f^o0–20 specific hypolipoproteinemic response to desiccated thyroid observed in the xanthoma tendinosum group is consistent with previously reported results obtained in euthyroid normals and in schizophrenic patients (Strisower *et al.*[10,12]). The large s_f^o0–20 hypolipoproteinemic response observed in the xanthoma tendinosum group was predictable, from our knowledge of the high correlations in other groups between initial s_f^o0–20 concentration and the decrease in s_f^o0–20 concentration with desiccated thyroid administration.

The s_f^o0–20 hypoplipoproteinemic response in xanthoma tendinosum to the administration of desiccated thyroid is of great practical importance to these individuals. Such patients are subject to early, severe, and often fatal, heart disease (Epstein *et al.*[3], Guravich[5], Harris-Jones, Jones and Wells[6]). Results of an extensive population follow-up study by Gofman *et al.*[4] have clearly indicated that individuals characterized by high lipoprotein and A.I. values are more prone to the development of clinical heart disease and its fatal consequences than individuals with low lipoprotein and A.I. values. It would be hoped that reduction of the elevated s_f^o0–20 serum lipoprotein concentrations in xanthoma tendinosum would lower very considerably the morbidity and mortality associated with coronary artery disease.

Summary. A group of 20 patients with the s_f^o0–20 hyperlipoproteinemia characteristic of xanthoma tendinosum was studied for an average of 2 years. Twelve of the 20 patients had the tendon lesions characteristic of xanthoma tendinosum.

The 20 patients were given desiccated thyroid (maximum average daily dose, 6 grains), for an average of 36 weeks.

Both the s_f^o0–12 and s_f^o12–20 low density lipoprotein concentrations were promptly and significantly reduced by the administration of desiccated thyroid, but no changes in the s_f^o20–100 or s_f^o 100–400 low density lipoprotein concentrations were observed. The Atherogenic Index (A.I.) and the serum cholesterol concentration were significantly

*The Atherogenic Index (AI) is defined by the formula:
AI = 0.1 (s_f^o0–12) + 0.175 (s_f^o12–400), where the concentrations are given in mg. per 100 ml.

†Xanthoma tendinosum is characterized by elevated concentrations of s_f^o20–100 and s_f^o100–400 serum lipoproteins (Gofman *et al.*[4]). In a small series of these patients, preliminary results indicate that the s_f^o20–100 and s_f^o100–400 serum lipoprotein concentrations were reduced as a result of the administration of 2 to 4 grains per day of desiccated thyroid.

reduced by the thyroid administration.

It would be hoped that reduction of the elevated $s_f^\circ 0–20$ serum lipoprotein concentrations in xanthoma tendinosum would lower very considerably the morbidity and mortality associated with coronary heart disease in these patients.

ACKNOWLEDGMENTS: We gratefully acknowledge the assistance of the members of the Ultracentrifugal Group at Donner Laboratory who performed the lipoprotein and cholesterol analyses. The Armour Laboratories, Kankakee, Illinois, supplied the desiccated thyroid used in this study.

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SUMMARIO IN INTERLINGUA

Le Effecto de Desiccate Extracto Thyroide Super in le Hyperlipoproteinemia de Xanthoma Tendinose

Un gruppo de 20 patientes con le hyperlipoproteinemia $s_f^\circ 0–20$ characteristic de xanthoma tendinose esseva studiate durante al media 2 annos. Dece-duo del 20 habeva le lesiones tendinal que es characteristic de xanthoma tendinose.

Le 20 patientes recipeva desiccate extracto thyroide in un dosage maximal de al media 6 granos per die durante un periodo medie de 36 septimanas.

Le concentrationes de lipoproteina del basse densitates $s_f^\circ 0–12$ e $s_f^\circ 12–20$ esseva prompte- e significativemente reducite per le administration de desiccate extracto thyroide, sed nulle alterationes in le concentration de lipoproteina del basse densitates $s_f^\circ 20–100$ o $s_f^\circ 100–400$ esseva observate. Le indice atherogene e le concentration seral de cholesterol esseva reducite significativemente per le administration thyroide.

Il es a sperar que le reduction del elevate concentrationes seral de lipoproteina $s_f^\circ 0–20$ in xanthoma tendinose reduce considerabilemente le morbiditate e mortalitate associate con coronari morbo cardiac in iste patientes.

THE EFFECT OF PARENTERAL VITAMIN B_{12} ON MUSCLE CONTRACTION IN HYPOTHYROIDISM*

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AND

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For many years it has been known that there is a relationship between the speed of muscle reflexes and the level of thyroid function (Mayo Clinic[7]). Most attempts at quantitation of the speed of muscle reflexes in thyroid disease have focused attention on muscle relaxation (Chaney[1], Harrell and Daniel[2], Houston[3], Lambert, Maderos and Kelsey[4]). This has been largely due to the type of recording devices used wherein the duration and speed-time patterns of muscle contraction could not be measured precisely in the monophasic curves produced.

We have recently developed a new device, the Kinemometer, which obviates some of the difficulties encountered by others, in that the free (isotonic) Achilles reflex response can be recorded as a diphasic curve in which contraction and relaxation are separately recorded with a high degree of precision. The actual instrumentation has been the subject of a previous publication (Lawson[5]).

During a study of over 3000 patients (Lawson[6]), it was noted accidentally that one of our patients who had received vitamin B_{12} had rather dramatic changes in the Achilles reflex. Further studies with vitamin B_{12} form the basis of this report.

Materials and Methods. The Kinemometer is in essence comparable to a velocity ballistocardiograph, in that it measures speed of movement. It differs from the traditional inductance ballistocardiograph in that it records movement primarily in only one plane, although the actual movement may have components in 3 planes. This is of some importance since the Achilles reflex movement, as obtained clinically, contains both lateral and vertical motion artefacts due to the hammer blow and these can affect the accuracy of measurement.

The free Achilles reflex is recorded as shown in Fig. 1. The segment of interest in the reflex tracing is the segment extending from the point of stimulation to the point at which the baseline is recrossed after the major downward deflection. This is known as the S-D segment. The duration or length of this segment has been found to correlate best with the patient's clinical status. It is highly reproducible and correlates best with end-organ response to thyroid hormone (Lawson[5]). Because of this we have abandoned measurement of any other interval or velocity measurement.

In this study 73 normal patients were observed after the parenteral administration of vitamin B_{12}. The criteria of normality being: no history of thyroid disease, no previous thyroid or iodide therapy, no menstrual irregularities, no palpable nodules or enlargement of the thyroid gland, a normal serum protein bound iodine (PBI) and a sense of physical well being.

Twenty-nine untreated hypothyroid patients were studied. The criteria of hypothyroidism being at least 2 abnormal tests among the following: serum PBI, I_{131} uptake, I_{131} conversion ratio, or basal metabolic rate.

*Presented before the Southern Section of the American Federation for Clinical Research, 22 January 1959, New Orleans, Louisiana.

Seven inadequately treated hypothyroid patients who fulfilled the criteria above at the initiation of therapy were studied, the criteria of undertreatment being unsatisfactory clinical response with subsequent sustained improvement on higher doses of thyroid. The beneficial results of increased dosage included electrocardiographic changes toward normal, more normal menses, improvement of anemia on thyroid alone, and disappearance of myxedema infiltrates which had not completely cleared on the dose of thyroid being administered.

Twelve patients who were felt to be overtreated with thyroid hormone were studied. The criterion of overtreatment being a sustained sense of clinical improvement at the lower dosage level.

Ten hyperthyroid patients were studied. Each of these patients had at least 2 of the following tests in the hyperthyroid range as well as good response to antithyroid therapy: an increased serum PBI, basal metabolic rate, I_{131} conversion ratio or uptake.

Results. In cases of myxedema it was noted that the S-D interval lengthened 40 milliseconds or more within 2 to 6 hours after the parenteral administration of 1 mg. of vitamin B_{12}. A typical reflex prolongation after B_{12} is shown in Fig 1.

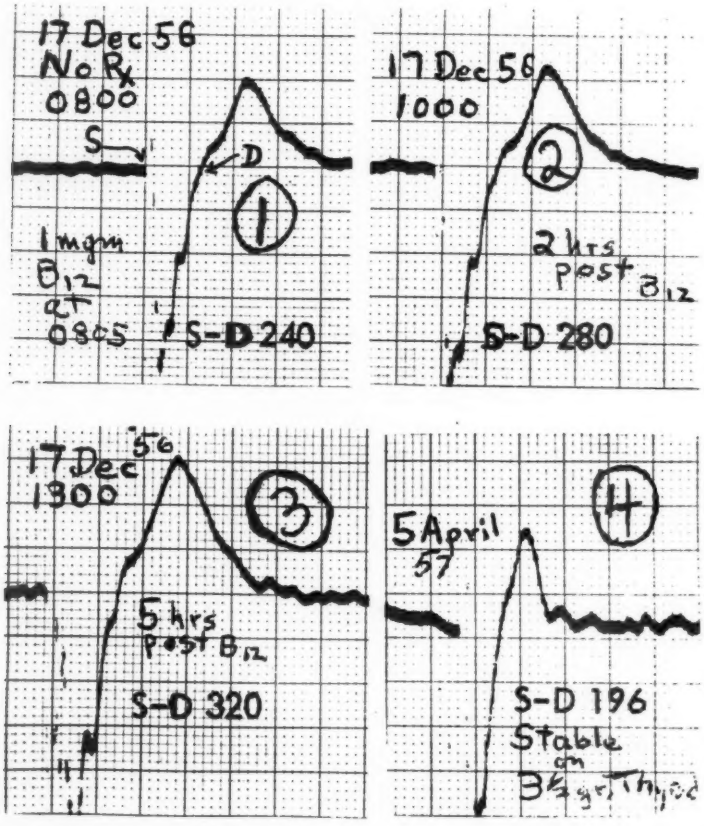

Fig. 1.—Typical response of a patient with untreated hypothyroidism to 1 mg. of vitamin B_{12} intramuscularly. Note the progressive lengthening of the Achilles reflex contraction time, as demonstrated by the widening of the S-D from 240 to 320 milliseconds, and eventual normalization which occurred 4 months after the institution of therapy.

In the 73 normal patients and 10 hyperthyroid patients there was no pro-

longation of the reflex after 1 mg. of vitamin B_{12}. The maximum variation in any patient was 16 milliseconds

In the 29 untreated hypothyroid patients and 7 inadequately treated hypothyroid patients there was an abrupt prolongation of the S-D segment of greater than 40 milliseconds within 6 hours after the administration of vitamin B_{12}. A typical response during treatment is shown in Fig. 2. This patient was switched to liothyronine* as part of another study and had not reached optimum thyroid dosage at the time that the B_{12} was administered. Her final liothyronine dose is roughly equivalent to 3 grains of thyroid extract.

In the 12 patients who were overtreated with thyroid, all showed an abrupt shortening of the Achilles reflex S-D within 4 hours after the administration of vitamin B_{12}. In every case the shortening was 20 milliseconds or more. Such a reaction can be seen at the point of B_{12} administration in Fig. 3.

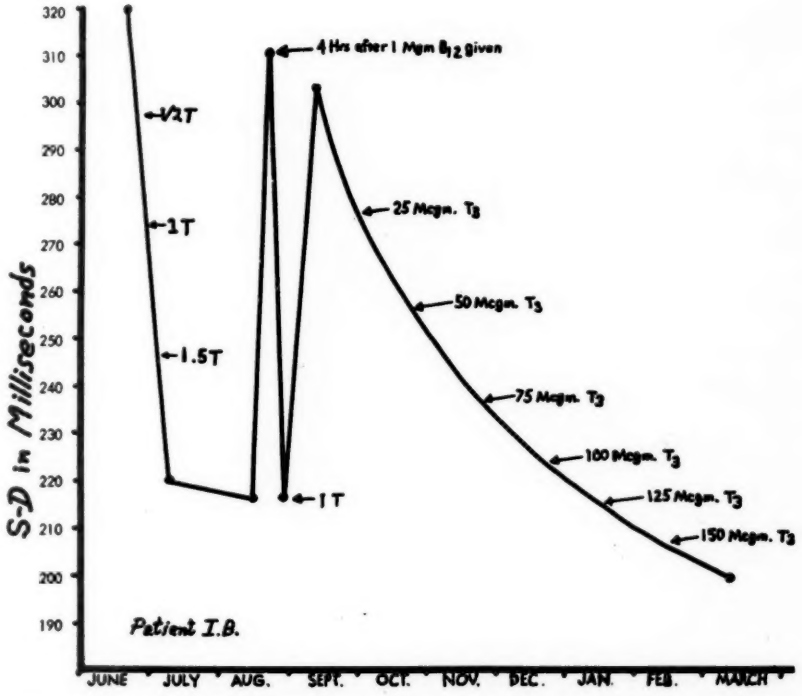

Fig. 2.—The S-D increased from 220 to 310 milliseconds 4 hours after 1 mg. of vitamin B_{12} intramuscularly, which is a reflection of inadequate dosage of thyroid extract.

It is interesting to note that there was a close and predictable response symptomatically to the administration of vitamin B_{12} depending on the patient's clinical status. In patients who were overtreated with thyroid and showed shortening of the S-D after the administration of B_{12} there was an amelioration of their symptoms within

*Available as Cytomel from Smith, Kline & French Laboratories, Philadelphia, Pa.

2 to 4 hours after the vitamin was given. In patients who were hypothyroid or undertreated, the administration of B_{12} caused an increase in their hypothyroid symptoms within 6 hours. The more severely hypothyroid patients complained rather strongly concerning the "bad feeling" following the vitamin. The symptomatic and reflex changes were well correlated as to degree and duration of response. The minimal period of reaction was 3 days and the maximal duration of symptomatic and reflex response following a single injection of vitamin B_{12} was 5 days.

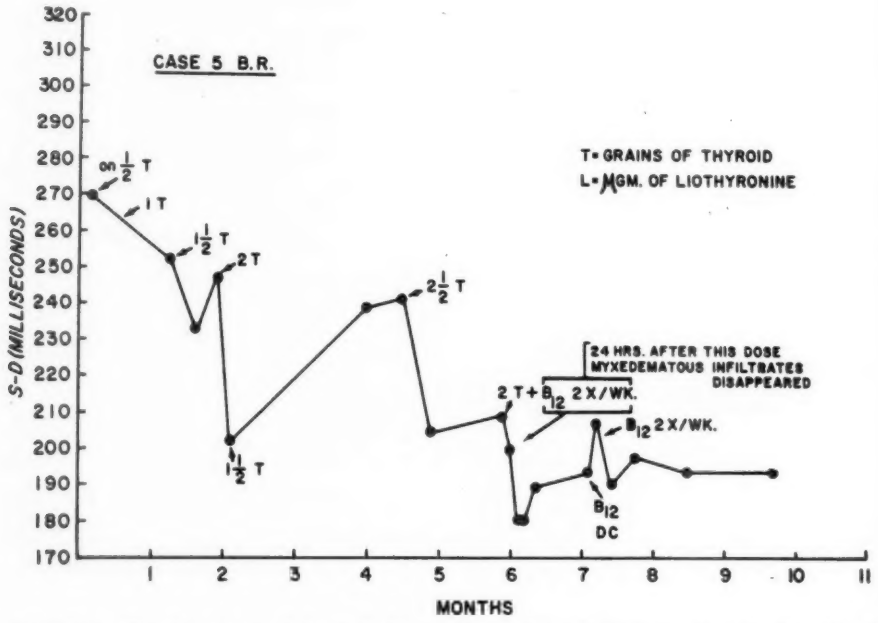

Fig. 3.—Note the shortening of the S-D from 200 to 180 milliseconds after the first injection of B_{12} demonstrating excessive dosage of thyroid. It was at this time that the patient's myxedematous infiltrates dramatically disappeared.

In Fig. 3 the reflex findings are presented in a case with a remarkable response to vitamin B_{12}. This 53-year-old myxedematous woman was seen in referral under the following circumstances. The patient initially had a PBI of 0.55 μg. per 100 ml. In the sixth month of therapy the patient's physician referred her to us, stating that each time her thyroid dosage had been increased to 2 grains or more of desiccated thyroid she had developed tachycardia, sweating, nervousness, insomnia and chest pain. He had repeated the PBI determination which now measured 6.8 μg. per 100 ml. He felt that she was therefore absorbing the medication, but her *myxedematous infiltrates had not disappeared on 2½ grains of thyroid.* Her electrocardiogram was still abnormal with low voltage, lowering of the P and T waves throughout, and sagging of the S-T segments across the left precordial leads. He was unsure as to what to do.

Upon seeing the patient, it was decided that it would probably be wise to reduce the patient's dose of thyroid,

and then give her an injection of vitamin B_{12} as a test since we had been successful with this technique in other cases which posed difficult problems. Approximately one week after the thyroid dosage had been reduced, the patient was given 1 mg. of vitamin B_{12} intramuscularly. The following day she reported to her physician, saying "I have been up all night going to the urinal." Fortunately, she had been weighed the previous day and when she was again weighed, it was noted that she had lost 8 pounds during the night. Her myxedematous infiltrates had disappeared completely. An electrocardiogram was taken which showed marked improvement in voltage, diminished S-T sagging and greater T wave amplitude.

Due to the remarkable clinical improvement the patient was then stabilized on 1½ grains of thyroid daily and given 1 mg. of vitamin B_{12} twice weekly. On this regimen she remained free of symptoms and peripheral edema. At the end of 3 months the B_{12} injections were discontinued with reappearance of the myxedematous infiltrates. The patient was then changed to liothyronine and within 3 weeks had become stabilized on 100 μg. per day. On this medication alone she became asymptomatic, lost her peripheral edema and no longer requires supplemental vitamin B_{12} during a 2-year period of observation.

Discussion. We are by no means advocating the routine use of vitamin B_{12} in the *treatment* of hypothyroidism. In over 50 cases of hypothyroidism seen in the past 3 years, we have only encountered one case in which vitamin B_{12} appeared therapeutically necessary and, as given in the history presented, this patient could be maintained on liothyronine alone. Even in this single case the Schilling test was normal.

Although we do not have a satisfactory explanation for all of the observations presented, there are several clinical applications which are pragmatically useful:

In patients who are problem cases in the diagnosis of hypothyroidism by the routine tests of thyroid function and where the usual diagnostic tests may be in conflict, if the injection of 1 mg. of vitamin B_{12} causes a significant prolongation of the Achilles reflex, the patients will usually be objectively benefited by thyroid therapy. We have seen 16 patients in the past 3 years.

The fact that one patient had such a remarkable diuresis following 1 mg. of vitamin B_{12} lends further support to the concept that, at least in *pharmacological* doses, vitamin B_{12} has some rather marked physiological actions in occasional cases of myxedema other than on the muscle reflex.

Another observation worthy of note is that the nervousness and irritability which occurs during treatment of hypothyroidism can occur as a result of either under or over dosage. This poses a therapeutic dilemma for the physician treating the patient. Are the symptoms due to too much or too little replacement therapy? When faced with this problem, we have had uniformly satisfactory results when using the muscle reflex changes following B_{12} administration as a guide. Generally, even without the reflex test, if the patient feels better, the dose of thyroid is too high; and if the symptoms become worse, the dose is too low. The main difficulty with such a clinical statement from the patient is that placebo effects can occur which cause confusion. In this regard it is interesting to note that the placebo responses that we have seen have usually not lasted more than one day, whereas the responses which correlated with objective muscle reflex changes lasted for at least 3 to 5 days.

Summary and Conclusions. The administration of 1 mg. of vitamin B_{12} to 73 euthyroid and 10 hyperthyroid patients caused no change in the Achilles reflex contraction time.

Twenty-nine untreated hypothyroid patients and 7 inadequately treated hypothyroid patients showed an abrupt, transient prolongation of the Achilles reflex contraction time of greater than 40 milliseconds following the parenteral administration of 1 mg. of vitamin B_{12}.

Twelve hypothyroid patients who were overtreated with thyroid had an abrupt shortening of their reflex contraction time of 20 milliseconds or more following the administration of vitamin B_{12}. Eight of the patients had previously shown prolongation at the time therapy was initiated.

We conclude that vitamin B_{12} has some unknown effect on the contractile process of skeletal muscle in patients who are hypothyroid and who are under or overtreated with exogenous thyroid. These patterns of muscular response following the administration of vitamin B_{12} have proved pragmatically useful in our hands when indecision as to the adequacy or inadequacy of thyroid dosage, or a problem in the diagnosis of hypothyroidism, has arisen.

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SUMMARIO IN INTERLINGUA

Le Effecto Exercite per Vitamina B_{12} in Administrationes Parenteral Super Le Contraction Muscular in Hypothyroidismo

Le administration de 1 mg de vitamina B_{12} a 73 subjectos euthyroide e a 10 patientes hyperthyroide causava nulle alteration del tempore de contraction in le reflexo achillee.

Vinti-novem non-tractate e 7 inadequatemente tractate patientes hypothyroide monstrava un abrupte sed transiente prolongation del tempore de contraction in le reflexo achillee, amontante a plus que 40 millisecundas, como effecto del administration parenteral de 1 mg de vitamina B_{12}.

Dece-duo patientes hypothyroide qui habeva recipite un excesso de tractamento con extracto thyroide monstrava un abrupte reduction del tempore de contraction in lor reflexos achillee, amontante a 20 millisecundas o plus, post le administration de vitamina B_{12}. Octo de iste patientes habeva previemente reagite per prolongation del tempore de contraction, *i.e.* al periodo quando le therapia esseva initiate.

Nos conclude que vitamina B_{12} exerce un incognoscite effecto super le processo de contraction del musculo skeletic in patientes qui es hypothyroide e qui es tractate inadequate- o excessivemente con exogene extracto thyroide. Iste modulation del responsa muscular post le administration de vitamina B_{12} se ha provate de utilitate pragmatic in nostre manos quando nos trovava nos indecise con respecto al adequatia o inadequatia de un dosage de extracto thyroide o in le presentia de un problema in le diagnose de hypothyroidismo.

THE HEART SOUNDS IN INTERMITTENT LEFT AND RIGHT BUNDLE BRANCH BLOCK

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The first and second heart sounds are due primarily to closure of the cardiac valves which are set in motion by pressure transmitted to the blood by myocardial contraction. It is not surprising therefore that bundle branch block, almost immediately after its earliest recognition, was reported to produce abnormalities in cardiac sounds (Eppinger and Stoerk[4]). Delay in electrical excitation with consequent delay in mechanical contraction is the present-day explanation of the abnormal relationship of the aortic and pulmonic components of the second sound when bundle branch block is present (Leatham[6]).

Normally, pulmonic valve closure follows aortic and the interval between the two increases with inspiration. An abnormally wide interval in right bundle branch block is attributed to pathologic delay in pulmonic valve closure secondary to pathologic delay in completion of right ventricular excitation. For similar reasons, delay in aortic valve closure when left bundle branch block is present is attributed to the block. In this situation, aortic valve closure may follow the pulmonic. Inspiration may then close the gap between the two.

Based upon this conceptual scheme, King[5] and others mistook the presystolic gallop sound for the first component of the first heart sound and consequently reported that the first heart sound was split in left bundle branch block. It is now agreed that the first heart sound is rarely split in bundle branch block (Leatham[6]). This apparently discrepant finding is explained by assuming that bundle branch block in the human is rarely complete but more or less peripheral. It has been shown that the onset of homolateral ventricular contraction is not delayed in human bundle branch block (Braunwald and Morrow[3]). There is likewise electrocardiographic evidence to support this view (Wada[15]).

There are nonetheless several inherent difficulties in determining the precise effects of bundle branch block on cardiac sound. There is the normal variation in the duration of intervals between the 2 components of the first and second sounds and also in the duration, frequency and amplitude of each component. There is also the fact

*Supported by the Heart Association of Southeastern Pennsylvania.

that left bundle branch block is most commonly associated with left ventricular disease. Under such circumstances, it may not be possible to separate the electrical effects on cardiac sounds from those due to myocardial disease.

Recently, we had the opportunity to study 2 patients with healed myocardial infarction and angina pectoris, one with intermittent left bundle branch block and the other with intermittent right bundle branch block. Our purpose is to describe the specific changes in cardiac sounds produced in these 2 patients by their conduction defects.

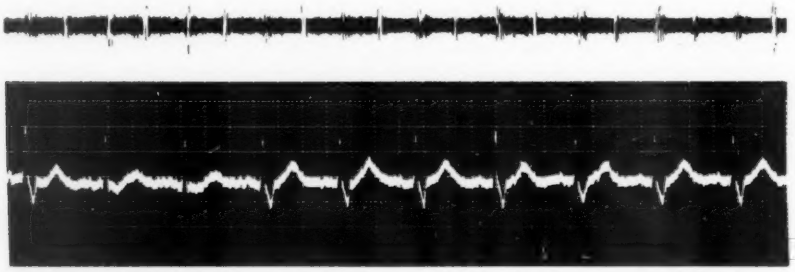

Fig. 1.—E.S. Female Age 35 yr. Old anterior myocardial infarction. Angina pectoris. Note changing character of first sound with change in ventricular conduction. Note also prolongation of mechanical systole of both ventricles and disproportionate delay in aortic component of second sound when left bundle branch block is present.

Case Reports.CASE 1. E.S., a 35-year-old woman, a nurse, was referred to one of us (L.A.S.) because of frequently recurring, and at times, completely incapacitating angina pectoris. Two years previously she had suffered a myocardial infarction immediately following a cholecystogram performed at another hospital to determine the cause of right upper quadrant abdominal pain. She was confined to that hospital for 2 months where conventional therapy for coronary thrombosis was instituted. Both during her stay in and since her discharge from that hospital, she had recurrent angina pectoris which, over the 2-year period, has increased in frequency and in duration.

Physical examination revealed a thin normally developed woman. The blood pressure was 132/74 mm. Hg. The entire physical examination was not remarkable except for auscultation.

Although the rhythm was completely regular, the first sound varied irregularly in intensity. At times, it was snappy and at other times dull. A systolic gallop was audible only when the first sound was intense. Inspiration slightly split the second sound.

The electrocardiogram confirmed the presence of an old anterior myocardial infarction. In addition, intermittent left bundle branch block was seen. A phonocardiogram was made to determine if the changing character of the heart sounds was related to changes in ventricular conduction.

The electrocardiogram (Fig. 1) showed a regular sinus rhythm and a constant PR interval undisturbed by the change in ventricular conduction. All measurements were determined by the use of accurate dividers.

Phonocardiograph (Fig. 1) confirmed the clinical observation of recurrent changes in the character of the first heart sound. It likewise revealed the intermittent intensity of a systolic gallop. Both these changes were constantly related to the type of ventricular conduction. The systolic gallop was of greater intensity when normal ventricular conduction was present.

QI (duration of Q to first vibrations of first sound) was constant regardless of the type of ventricular conduction. With normal ventricular conduction, the first sound was of high amplitude with its 2 major components clearly seen. With left bundle branch block, the 2 major components were absent and the amplitude of the first sound was considerably diminished so that its entire character was grossly distorted. With left bundle branch block, electrical systole was prolonged at the expense of electrical diastole. At the same time, mechanical systole of both the right and left ventricles was prolonged; the left more so than the right so that aortic and pulmonic valve closure coincided.

CASE 2. C.W. a 56-year-old man, a grocer, was referred to one of us (L.A.S.) because of recurrent angina pectoris and shortness of breath. He had suffered a myocardial infarction 10 years previously. He recovered completely and was free of symptoms for 8 years. He then developed angina pectoris and, one and a half years later, symptoms and signs of congestive failure. He became progressively worse over the next 6 months.

Physical examination revealed an obviously sick man in congestive heart failure. The blood pressure was 160/100 mm. Hg. The heart was moderately enlarged due predominantly to the left ventricle. Although the rhythm was regular, the first sound varied in intensity. Inspiration made the minimal split of the second sound more obvious.

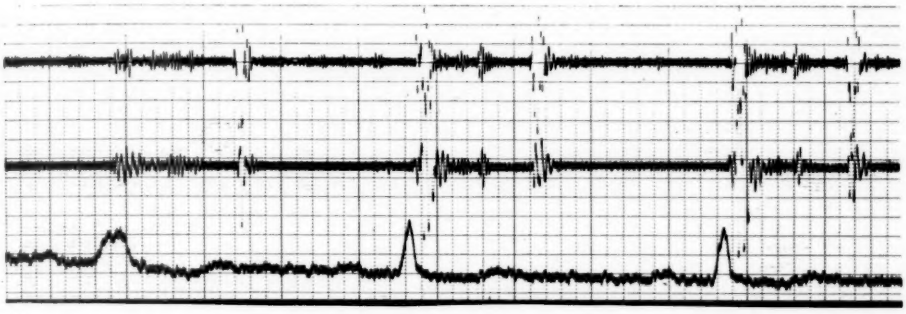

Fig. 2.—S.W. Male 56 yr. Old myocardial infarction. Angina pectoris. Note changing character of first heart sound with change in ventricular conduction. Note also prolongation of mechanical systole of both ventricles but no change in interval between the two components of the second sound when right bundle branch block is present.

The electrocardiogram confirmed the presence of an old myocardial infarction and also revealed intermittent right bundle branch block.

A phonocardiogram was done to determine whether the changing character of the first sound was due to the intermittent block.

The electrocardiogram (Fig. 2) again revealed a constant rhythm and a PR interval undisturbed by the changing ventricular conduction.

The QI interval was constant regardless of ventricular conduction. With normal ventricular conduction, the first sound was of high amplitude with both major components clearly visible. With right bundle branch block, both major components were absent and the amplitude of the first sound diminished greatly, changing its entire contour. At the same time, electrical systole was prolonged at the expense of diastole. Mechanical systole of both ventricles was equally prolonged so that there was no measurable change in the interval between the 2 components of the second sound.

Discussion. Wiggers[16] demonstrated that a change in intensity of the first sound in a given area is a reliable indication of change in the rate of rise of pressure during the isometric contraction phase. The change in intensity of the first sound with changes in cardiac rate and in the PR interval (Wolferth and Margolies[17]) are secondary to an obligatory change in the rate of rise of pressure. In our 2 cases, the PR interval, cardiac rate and blood pressure were constant. Yet intermittent block on the right as well as that on the left side abolished the snap of the first heart sound. Our phonocardiographic findings when block was present are similar to those described by Battro, Braun-Menendez and Orias[1] who called these contours fractionization of the vibrations. They explained these findings on the basis of a concept of the origin of the first heart sound dominant at that time. The first component was regarded as due to simultaneous closure of the mitral and tricuspid valves and the second to simultaneous opening of the aortic and

pulmonary valves. With normal conduction, each component represented a summation of 2 simultaneous events. In bundle branch block, contraction of the ventricles was asynchronous and, therefore, fractionization of the sounds occurred, the degree varying from case to case depending upon the degree of asynchronism.

Such an explanation is no longer tenable because the 2 components of the first heart sound are regarded as produced by mitral and tricuspid valve closure in succession (Leatham[6]). The change in both components of the first heart sound in our 2 cases suggests that bundle branch block, whether right or left, changed the initial rising tension in both ventricles. How can this occur?

All students agree that initial electrical activity of the heart spreads to the septum, a structure that is common to both ventricles. It is not certain whether the septum is composed of a constrictor muscle which is distributed unequally to each ventricle (Rushmer[11]) or whether it is formed by 2 separate but intimately attached muscle bundles, one supplying the left and the other the right ventricle (Medrano *et al.*[8]). In the former event, electrical excitation of the septum must initiate contraction in both ventricles. In the latter, the phenomenon of mechanical synchronization should operate. Seegers[12] demonstrated synchronization of juxtaposed myocardial tissues both for mechanical and electrical events. With either concept of septal architecture, unilateral bundle branch block would impair initial myocardial contraction in each ventricle. Such a phenomenon could explain the diminished intensity of both components of the first sound as well as Braunwald and Morrow's[3] observations of a lack of significant asynchronous contractions of the ventricles in left bundle branch block and in the majority of cases with right bundle branch block. These observations are also in keeping with the blood supply of the conduction septum[7]. They are consistent with experimental evidence that even complete occlusion of one of the 2 vessels supplying the conduction septum permits the persistence of viable fiber groups of conduction tissue (Lumb, Shacklett and Dawkins[7]). If both vessels are occluded, death occurs unless an additional blood supply to the septum is present. In this latter instance, viable fibers of conduction tissue are also present (Lumb, Shacklett and Dawkins[7]). On the other hand, the concept that bundle branch block represents solely peripheral block is incompatible with diminution of intensity of both components of the first heart sound.

That there is a definite change in contraction of both ventricles in our cases of bundle branch block, even though there is no significant difference in onset of contraction, is clearly indicated by the fact that mechanical systole in each ventricle is longer when bundle branch block is present than when normal ventricular conduction prevails. This observation is in keeping with histologic evidence that electrocardiograms with unilateral bundle branch block are derived from hearts with bilateral bundle branch disease (Yater[19]).

Left bundle branch block produced a disproportionate delay in aortic valve closure whereas right bundle branch block produced no change in the interval between the components of the second sound. Both patients had predominant left ventricular disease. It is possible that the extent and location of myocardial disease rather than bundle branch block may be the more important factor in determining the characteristics of the second sound when bundle branch block is present.

That mechanical factors independent of electrical ones are important determinants of the second sound is well known. Boyer and Chisholm[2] have shown that inspiration shortens left ventricular as well as prolonging right ventricular mechanical systole. Perloff and Harvey[9] have shown that the widely split second sound in atrial septal defect disappears after its correction even though the pattern of incomplete right bundle branch block persists. We have observed the appearance of right bundle branch block after repair of a ventricular septal defect without change in temporal relationship of pulmonic and aortic valve closure (Soloff[13]). The effects on cardiac sound of mitral regurgitation, pulmonic and aortic stenosis and patent ductus arteriosus as well as of other mechanical disturbances are all well known. An abnormal second sound in congenital right bundle branch block could be due to anomalous myocardial septal architecture. We are unaware of any studies on the nature of the septal myocardium in congenital block. That the anatomic partition of the septum depends upon the degree and location of acquired heart disease has been demonstrated by the studies of Bonetti and Lev (quoted by Sodi-Pollares[8]).

This study indicates that the precise effects of bundle branch block on cardiac sound cannot be determined when both myocardial disease and bundle branch block are irreversibly present together. Only a study of a large number of cases with intermittent bundle branch block will isolate electrical from myocardial causes of abnormalities in cardiac sound

Finally, our first case illustrates that systolic gallop, distinct from systolic clicks or extracardiac sounds, can occur. This phenomenon was reaffirmed by Wolferth and Margolies[17], although its existence has been denied by many subsequent students (Thomson and Levine[14]).

Summary. 1) The phonocardiographic findings of 2 persons with coronary artery disease, one with intermittent left, and the other with intermittent right bundle branch block, are described. 2) Both major components of the first sound were absent when either left or right bundle branch block was present. 3) Mechanical systole of both ventricles was prolonged when either form of block was present. 4) Homolateral disproportionate prolongation of mechanical systole occurred only when bundle branch block was confined to the more diseased ventricle. 5) An explanation of these findings is suggested. 6) Incidentally, the existence of a systolic gallop distinct from systolic click or extracardiac sound, is reaffirmed.

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SUMMARIO IN INTERLINGUA

Le Sonos Cardiac in Intermittente Bloco de Branca Sinistre e Dextere

1. Es describite le constatationes phonocardiographic in 2 subjectos con morbo de arteria coronari e intermittente bloco de branca, le un sinistre, le altere dextere.

2. Ambe le major componentes del prime sono esseva absente in le presentia tanto de bloco de branca sinistre como etiam de bloco de branca dextere.

3. Systole mechanic de ambe ventriculos esseva prolongate in le presentia del un e del altere forma de bloco.

4. Un disproportionate prolongation homolateral del systole mechanic occurreva solmente quando le bloco de branca esseva confinate al plus afficite ventriculo.

5. Un explication de iste constatationes es proponite.

6. A proposito, le existentia de un galopo systolic distincte ab le clic systolic o le sono extracardiac es reaffirmate.

THE EFFECT OF PYRAZINAMIDE-INDUCED HYPERURICEMIA ON SERUM LIPIDS

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Atherosclerosis has long been known to be associated with high serum uric acid levels and to be a frequent complication of gout (Bauer and Klemperer[3]). Investigating this, Gertler, Garn and Levine[7] measured serum uric acid levels in patients with coronary artery disease and found that 24% of the group studied had levels of serum uric acid over 6.0 mg. per 100 ml. as compared to 6% of the normal control group. They also found the mean uric acid level significantly higher in the coronary artery disease group, and a large number of patients had both abnormally high uric acid and cholesterol. These findings give rise to a question of a causative relationship of hyperuricemia to hypercholesterolemia and other lipid disturbances of the blood.

The antituberculosis drug, pyrazinamide, is known to cause an elevation of serum uric acid after a few days administration (Am. Trudeau Soc.[2], Cullen, Early and Fiore[5], Gleason, Street and Kahn[8], Kanner and Jacobs[10], Weiss *et al.*[11]). This elevation is observed regardless of the premedication level of the patient's serum uric acid. Also, the serum uric acid returns to its premedication level when administration of the drug is stopped. This pyrazinamide effect on the uric acid of the blood presented an opportunity to study what effect an induced hyperuricemia would have on the various blood lipids. It appeared to be a suitable method of studying lipid changes under conditions where the uric acid level is the only variable factor.

Method of Study. Ten subjects who were hospitalized with active tuberculosis were given pyrazinamide orally in a dosage of 2 gm. per day for a period of 1 to 4 weeks. Blood serum was analyzed for uric acid, total cholesterol, lipid phosphorus and alpha and beta lipoproteins. This was done before pyrazinamide was administered, during the administration of the drug, and after the drug was stopped. The control or pre-drug values were obtained by using the mean of 3 separate determinations at approximately 1 week intervals. Blood samples were drawn with the patient in the preabsorptive state and all analyses were made in duplicate.

Serum uric acid was determined by the method of Brown[4]. Total serum cholesterol was obtained by a modification of the method of Abell *et al.*[1]. Lipid phosphorus was obtained by the method of Youngburg and Youngburg[12], using the phosphate procedure of Fiske and Subbarow[6]. Lipoproteins were determined by a modification of the method of Jencks and Durrum[9].

Results. Table 1 shows the serum uric acid and lipid values before, during and after pyrazinamide administration of all the subjects studied. Hyperuricemia developed in all patients after 4 to 7 days administration of the drug and was maintained for periods of 3 days to 3 weeks. There was a mean elevation of 83% in the serum uric acid. Within one week after stopping pyrazinamide, the uric acid returned to approximately the pretreatment level in all instances.

TABLE 1.—SERUM URIC ACID, TOTAL CHOLESTEROL, PHOSPHOLIPID AND BETA LIPOPROTEIN LEVELS IN PATIENTS BEFORE, DURING AND AFTER PYRAZINAMIDE ADMINISTRATION

Subject	*Age*	*Sex*		*Uric Acid mg. per 100 ml.*	*Total Cholesterol mg. per 100 ml.*	*Phospholipid mg. per 100 ml.*	*Beta Lipoprotein %*
1	32	F	Before drug	3.8	170	223	76.4
			During "	7.0	162	198	78.4
			After "	3.9	152	195	72.4
2	23	F	Before drug	3.5	186	196	79.2
			During "	6.4	178	175	74.2
			After "	4.0	169	185	78.6
3	51	F	Before drug	3.7	216	264	77.7
			During "	7.7	215	233	75.9
			After "	4.0	200	226	83.8
4	46	M	Before drug	6.0	272	313	85.9
			During "	10.4	195	217	80.4
			After "	7.6	224	268	84.2
5	29	M	Before drug	5.3	152	169	75.7
			During "	10.3	151	195	77.4
6	33	M	Before drug	7.1	256	265	80.8
			During "	12.6	252	292	78.7
			After "	6.1	222	295	75.4
7	54	M	Before drug	7.8	294	280	87.1
			During "	16.5	289	312	86.3
			After "	5.5	259	312	88.9
8	34	M	Before drug	5.0	160	218	70.6
			During "	7.2	179	220	78.9
			After "	4.1	188	245	68.4
9	42	M	Before drug	5.3	162	195	76.2
			During "	7.8	174	203	78.9
			After "	5.4	189	195	78.2
10	37	M	Before drug	3.7	170	213	71.4
			During "	7.6	196	222	67.5

Nine of the 10 subjects developed no significant change in serum cholesterol, phospholipid or lipoprotein during the period of hyperuricemia as compared to the lipid levels during the lower serum uric acid period before pyrazinamide was given. One subject showed a significant drop in cholesterol and phospholipid during hyperuricemia, but the lipids did not return to their control levels when the drug was stopped and the serum uric acid returned to its pre-pyrazinamide level. Two patients had abnormally high control uric acid levels and developed marked hyperuricemia after pyrazinamide administration; however, no significant serum lipid changes occurred. Probenecid in a dosage of 2 gm. per day was given to 2 subjects during the period of hyperuricemia. This resulted in a reduction in the serum uric acid, but not to the pretreatment level. The serum lipids were not affected.

Discussion. The observations made

in this study indicate that hyperuricemia induced by the drug pyrazinamide in patients with active tuberculosis does not cause an increase in serum lipids. This at least suggests that in an individual with both hyperuricemia and hypercholesterolemia, the high level of uric acid in the serum does not in itself cause the high level of cholesterol. Rather, the simultaneous presence of these 2 states is probably the result of separate disturbances of uric acid and cholesterol handling which may have a modifying, but not an etiological relation to each other.

Summary and Conclusions. Hyperuricemia was induced in 10 subjects with active tuberculosis by the administration of pyrazinamide, and the effect on serum lipids was studied. Nine had no significant change in lipids, and one subject developed a lower serum cholesterol and phospholipid level during hyperuricemia.

The findings suggest that in patients with induced hyperuricemia, the high uric acid content of the blood does not cause an elevated cholesterol level or other lipid disturbances.

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SUMMARIO IN INTERLINGUA

Le Effecto Execite Super le Lipidos del Sero per un Hyperuricemia Inducite per Pyrazinamido

In 10 subjectos con tuberculose active, hyperuricemia esseva inducite per le administration de pyrazinamido, e le effectos del droga super le lipidos del sero esseva studiate. Novem del subjectos monstrava nulle significative alteration lipidic. Le decime disveloppava un reducite nivello seral de cholesterol e phospholipido durante le hyperuricemia.

Le constatationes de iste studio pare indicar que in patientes con hyperuricemia inducite, le alte contento de acido uric in le sanguine non resulta in un elevate nivello de cholesterol o in altere disturbationes lipidic in le sero.

STUDIES ON THE ABSORPTION, DISTRIBUTION, EXCRETION, AND USE OF A NEW LONG-ACTING SULFONAMIDE (SULFADIMETHOXINE) IN CHILDREN AND IN ADULTS

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A new long-acting sulfonamide, sulfadimethoxine (4 sulfanilmide-2, 6 dimethoxypyrimidine)* has recently become available for clinical investigation. Experimental observations indicate that it has an antibacterial effect in animals comparable to that of other sulfonamides (Schnitzer *et al.*[4]).Pharmacologic data obtained from animal studies with sulfadimethoxine are particularly interesting. It appears to be rapidly and almost quantitatively absorbed following oral administration. In the blood it exists predominantly (90%) as the free or therapeutically effective form (Randall, Bagdon and Engelberg[3]). Appropriate single oral doses produce adequate therapeutic levels in the blood for 24 to 36 hours. Excretion is by way of the kidney where only a small proportion (15 to 25%) is present in the acetylated form. Free sulfadimethoxine and its metabolites are reasonably soluble in urine within the usual physiologic pH range of urine (Boger[1]).

It was therefore felt to be of some importance to extend the studies of the absorption, distribution, penetration and excretion of sulfadimethoxine in children and adults and to examine the therapeutic efficacy and side effects associated with its clinical use.

Material and Methods. Whole blood free sulfonamide levels were determined by the Bratton-Marshall method, using 0.1 ml. samples of whole blood. Free and total sulfonamide in urine was determined by the same technique before and after acid hydrolysis. Trichloracetic acid filtrates were used throughout. Crystalline sulfadimethoxine served as a reference standard for establishing absorption curves, and in addition a standard was included with each series of determinations. The time specimens were obtained as indicated in the individual tables (1 to 6). Blood specimens were obtained by capillary puncture. Urine specimens were obtained by a spontaneous voiding. Control blood and urines were included in each series of studies.

The absorption, distribution and excretion of sulfadimethoxine were evaluated in children and in adults hospitalized on the pediatric and medical wards of Jefferson Davis Hospital. The children ranged in age from 3 months to 12 years and in weight from 3.5 kg. to 31 kg. The adult subjects varied in age from 16 to 76 years and from 27 kg. to 91 kg. in weight. There were approximately equal numbers of males and females in both age groups.

*Madribon. Hoffman-La Roche Inc.

TABLE I.—FREE SULFONAMIDE LEVELS IN WHOLE BLOOD FOLLOWING THE ADMINISTRATION OF SINGLE ORAL DOSES OF SULFADIMETHOXINE TO CHILDREN IN SUSPENSION.

No. of Pts.	*Av. Age*	*Av. Wt. (kg.)*	*Av. S.A. (M^2)*	*Dose gm./M^2*	*Free Sulfonamide Levels (mg. per 100 ml.)*					
					3 hrs.	*6 hrs.*	*9 hrs.*	*12 hrs.*	*24 hrs.*	*48 hrs.*
5	7.2	24.6	0.9	.5	7.5 (6.2–8.4)*	6.6 (5.7–7.8)	5.1 (4.0–7.0)	4.8 (3.4–6.1)	3.4 (1.9–5.0)	1.4 (0–3.7)
5	3.4	15.6	.63	1.0	17.4 (10.9–23.8)	15.0 (8.9–18.8)	13.4 (8.0–18.4)	9.1 (5.3–12.4)	7.4 (4.1–11.3)	4.1 (2.0–8.0)
6	26	10.4	.45	1.5	15.9 (12.1–20.9)	16.1 (14.0–19.7)	14.8 (9.2–18.5)	13.2 (12.1–14.5)	9.6 (7.8–11.4)	3.8 (2.6–4.6)

*Figures in parenthesis represent minimum and maximum levels.

TABLE 2.—FREE SULFONAMIDE LEVELS IN WHOLE BLOOD FOLLOWING THE ADMINISTRATION OF SINGLE ORAL DOSES OF SULFADIMETHOXINE TO CHILDREN IN TABLET FORM.

No. of Pts.	*Av. Age*	*Av. Wt.*	*Av. S.A. (M^2)*	*Dose gm./M^2*	*Free Sulfonamide Levels (mg. per 100 ml.)*									
					1 hr.	*2 hrs.*	*3 hrs.*	*6 hrs.*	*9 hrs.*	*12 hrs.*	*24 hrs.*	*36 hrs.*	*48 hrs.*	*60 hrs.*
5	6.2	19.0	.77	1.0	3.4 (1.6–* 6.1)	5.4 (1.9–8.3)	5.8 (1.6–9.0)	7.8 (6.1–9.9)	7.9 (6.3–12.8)	8.5 (5.9–13.4)	6.0 (3.8–8.8)	5.4 (2.9–9.2)	2.1 (0.8–4.1)	1.6 (0.8–2.4)
6	5.7	19.8	.80	1.5	2.0 (0–3.3)	3.5 (1.5–4.8)	5.9 (3.8–10.7)	8.2 (5.9–14.7)	9.4 (4.3–13.0)	11.3 (3.5–18.2)	6.7 (2.7–11.0)	—	3.7 (1.2–6.1)	—

*Figures in parenthesis represent minimum and maximum levels.

TABLE 3.—EXCRETION OF FREE AND TOTAL SULFONAMIDE IN THE URINE FOLLOWING THE ORAL ADMINISTRATION OF SULFADIMETHOXINE.

Patient	Wt. kg.	S.A. M^2	Dose gm.		Urinary Excretion of Sulfonamide—mg. 0–12 hours	12–24 hours	24–36 hours	36–48 hours	48–60 hours	60–72 hours	72–84 hours	84–96 hours
1.	20	.86	1.0	free	19	100	260	114	72	35	19	13
				total	20	138	462	133	94	45	18	40
2.	25	.90	1.0	free	100	157	165	98	56	84	47	7
				total	122	200	229	98	84	73	162	52
3.	20	.80	1.0	free	22	153	165	104	64	44	—	21
				total	152	203	247	119	67	51	33	22
4.	22	.87	1.0	free	88	115	95	105	95	30	27	20
				total	110	148	132	123	130	36	27	20
5.	16	.70	*0.5	free	79	67	92	31	20	—	39	20
				total	113	140	194	53	22	—	41	21
6.	30	1.20	*1.5	free	164	163	147	93	122	26	40	31
				total	258	324	333	202	203	36	89	80

*Suspension form used (all others tablets).

TABLE 4.—FREE SULFADIMETHOXINE LEVELS IN WHOLE BLOOD IN CHILDREN RECEIVING MULTIPLE ORAL DOSES IN 10% SUSPENSION AND TABLET FORM 0.5 GM. PER M^2 INITIALLY FOLLOWED BY 0.25 GM. PER M^2 AT 24-HOUR INTERVALS.

No. of Pts.	Av. Age	Av. Wt.	Av. S.A.	Dose Form	Free Sulfonamide Level—(mg. per 100 ml.) 6	12	24†	30	48†	54	72	78
12	5½	19.1 kg.	0.8 M^2	*Susp.*	7.7 (5.8–11.7)*	6.2 (4.0–9.5)	4.8 (3.2–7.0)	8.1 (5.3–13.5)	4.4 (2.9–11.2)	7.5 (6.2–9.9)	4.6 (3.0–7.4)	7.7 (5.5–11.3)
6	7	21.9	0.86	Tablets	7.7 (5.2–11.4)	6.0 (4.9–7.2)	4.0 (2.3–6.2)	5.7 (3.7–9.9)	3.7 (1.7–7.5)	5.5 (3.1–11.7)	3.6 (1.4–9.0)	6.3 (4.1–12.0)

*Figures in parenthesis represent minimum and maximum levels.
†Dose given following this blood specimen.

TABLE 5.—FREE SULFONAMIDE LEVELS IN WHOLE BLOOD IN ADULTS AFTER INGESTION OF SINGLE ORAL DOSE (TABLET).

No. of Pts.	*Av. Age*	*Av. Wt.*	*Dose*	*Free sulfonamide levels—(mg. per 100 ml.)* 2 hrs.	4 hrs.	8 hrs.	12 hrs.	24 hrs.	48 hrs.
5	43	78 kg.	1.0 gm.	3.0 (1.0–6.4)*	3.3 (1.5–6.2)	3.3 (1.9–4.6)	3.4 (2.1–4.3)	2.5 (1.6–3.4)	1.0 (0.7–1.5)
6	50	52 kg.	2.0 gm.	5.1 (2.0–6.4)	6.9 (5.4–8.6)	6.8 (4.8–8.9)	6.7 (5.1–8.1)	5.1 (3.7–6.0)	3.5 (2.7–4.6)
8	41	63 kg.	3.0 gm.	6.9 (5.0–12.6)	9.0 (5.1–15.0)	9.2 (5.2–15.2)	7.9 (5.6–9.4)	6.9 (4.4–8.7)	4.4 (2.2–6.7)

*Figures in parenthesis represent maximum and minimum level.

TABLE 6.—BLOOD FREE SULFONAMIDE LEVELS IN ADULTS FOLLOWING THE ORAL INGESTION OF VARYING DOSES OF SULFADIMETHOXINE FOR 4 DAYS.

No. of Pts.	*Av. Age*	*Av. Wt.*	*Initial Dose*	*Daily Dose*	*Blood free sulfonamide level (mg. per 100 ml.)* 2 hrs.	4 hrs.	6 hrs.	8 hrs.	12 hrs.	24 hrs.	30 hrs.	48 hrs.	54 hrs.	72 hrs.	78 hrs.
6	41	64 kg.	0	2.0 gms.	3.6 (1.7–8.0)*	6.0 (3.0–9.3)	—	5.8 (3.0–8.1)	6.0 (3.2–8.1)	5.0 (6.8–8.1)	11.2 (6.8–16.6)	7.9 (5.8–13.6)	10.7 (9.0–12.7)	7.3 (6.8–7.9)	12.0 (10.8–13.9)
4	26	76.5 kg.	2.0 gms.	1.0 gms.	—	—	6.6 (3.7–8.8)	—	—	4.6 (3.2–5.2)	8.8 (7.5–10.8)	5.7 (4.5–7.5)	8.1 (5.9–10.8)	5.8 (3.9–7.3)	9.1 (7.3–11.5)
4	33	58 kg.	2.0 gms.	1.5 gm.	—	—	8.4 (5.5–8.7)	—	—	5.5 (5.1–6.1)	11.3 (9.2–13.4)	8.0 (5.9–9.6)	13.6 (11.3–16.2)	9.3 (7.0–12.0)	9.5 (8.0–11.3)
3	60	56 kg.	3.0 gms.	2.0 gms.	4.7 (2.9–6.3)	7.2 (5.1–8.7)	—	7.6 (5.1–9.3)	7.9 (5.6–9.4)	8.0 (7.6–8.7)	9.2 (7.6–11.1)	10.0 (8.0–12.3)	13.1 (11.1–16.9)	11.1 (9.1–13.1)	12.8 (11.2–14.3)

*Figures in parenthesis represent minimum and maximum level.

In the absorption studies the initial dose was given while the patient was in a fasting state. Penetration studies into the cerebrospinal fluid were carried out only when the patient required lumbar puncture (or other procedure) as a part of his general diagnostic or therapeutic plan.

The therapeutic efficacy and side effects were studied in some detail in 369 children. The age range in this group varied from 3 months of age to 13 years of age. All patients were observed for skin manifestations and gastrointestinal symptoms. Twenty-two patients were treated in the hospital and had blood counts at the time the drug was started, at 5- to 7-day intervals, and after treatment. This same group of 22 patients was also followed for renal side effects with urinalyses (base line, twice weekly, after treatment). The 347 consecutive patients treated on an outpatient basis were observed for evidence of intolerance and side effects to sulfadimethoxine.

Results. CHILDREN. The administration of sulfonamides to patients of widely variable size necessitates that the dose be proportioned to the mass of the subject. This is usually accomplished by giving medications on the basis of quantity per kilogram of body weight. More recently Crawford, Kenny and Rourke[2] have shown that the dosage of sulfonamides may be estimated conveniently on the basis of surface area and our previous experience substantiated this observation.

Table 1 records the blood free sulfonamide levels obtained following single oral doses of the 10% suspension of sulfadimethoxine at dose levels of 0.5, 1.0, and 1.5 gm. per M^2 surface area. The maximum level in the blood was in 3 hours in patients receiving the suspension form and a satisfactory level persisted for 8 hours with the 0.5 gm. per M^2 dose, for 36 hours with the 1.0 gm. per M^2 dose, and for 48 hours with the 1.5 gm. per M^2 dose. In general, the larger doses tend to produce more sustained blood free sulfonamide levels but not always proportionately higher blood levels.

Table 2 records the free sulfonamide levels in children following the administration of sulfadimethoxine at 1.0 and 1.5 gm. per M^2 surface area doses, utilizing the 0.5 gm. tablet dosage form. The data for the 0.5 gm. per M^2 single dose is taken from the first 24 hours data of Table 4. Peak blood levels were achieved by the sixth hour for the 0.5 gm. per M^2 dose and by the twelfth hour for the higher dose levels. Satisfactory blood free sulfonamide levels persisted for 12 hours at the 0.5 gm. per M^2 dose level and for 36 hours for both the higher dose levels. It should be noted that the quantity of sulfonamide given in Tables 1 and 2 is similar and that only the dosage form is different. These data indicate that while the blood free sulfonamide levels are similar following the 0.5 gm. dose in both tablet and suspension form, the suspension form is more rapidly absorbed when given in 1.0 and 1.5 gm. per M^2 doses and produces a higher blood level than a similar dose in tablet form. The data recorded in Tables 1 and 2 are presented graphically in Fig. 1.

The free and total sulfonamide excretion in the urine of 6 children over a 4-day period after receiving single oral doses ranging from 0.5 to 1.5 gm. of sulfadimethoxine are presented in Table 3 and Fig. 2. The quantity excreted in the urine is recorded per 12-hour period and is not cumulative.

On the basis of the single dose absorption studies in children plus data available from multiple dose absorption studies in adult subjects, it appeared that satisfactory blood levels of free sulfonamide could be maintained with a schedule of 0.5 gm. per M^2 per day initially, then 0.25 gm. per M^2 per day thereafter. Table 4 records the data obtained in studies of this dosage schedule for both the suspension and the tablet form. In general the levels following the suspen-

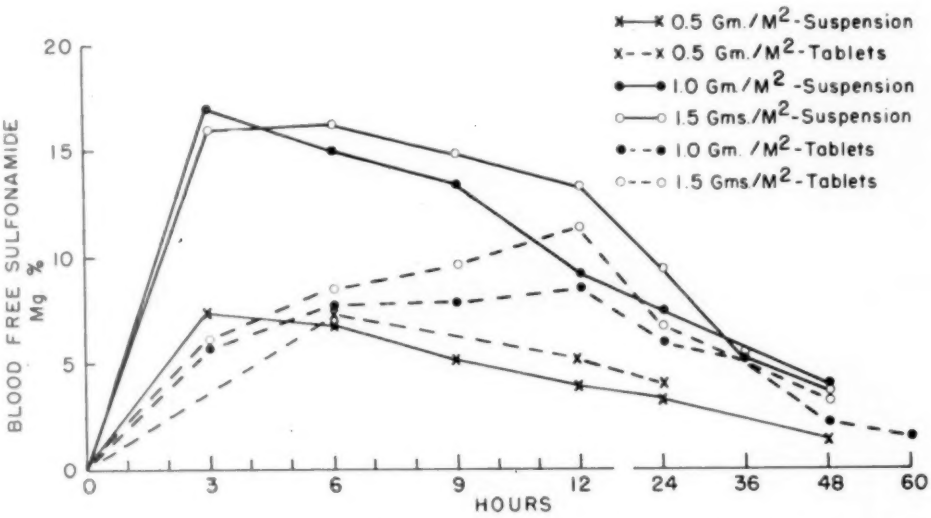

Fig. 1.—Comparison of the average blood free sulfonamide levels in children following the oral administration of sulfadimethoxine in 2 dosage forms and at 3 dosage levels (mean values from Tables 1 and 2).

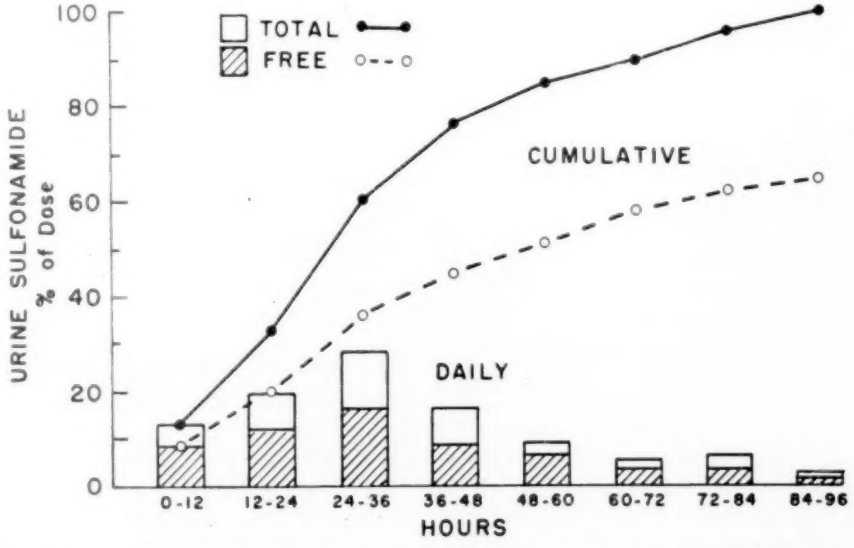

Fig. 2.—The average pattern of urinary excretion of free and total sulfonamide following the oral administration of sulfadimethoxine to 6 children. The bar graphs represent the excretion per period and the line graph the cumulative excretion (mean values from Table 3).

sion dosage form were more nearly ideal than the levels following the tablet dosage form though both schedules could probably be considered adequate.

ADULTS. The administration of single oral doses of sulfadimethoxine to adult subjects at levels of 1.0, 2.0 and 3.0 gm. single oral doses is recorded in Table 5. A peak level is reached at 4 hours following the 1.0 gm. dose and the 2.0 gm. dose, and at 6 to 9 hours following the 3.0 dose. Average blood free sulfonamide levels above 5.0 mg.% were achieved only following 2.0 and 3.0 gm. doses.

Various dosage plans were studied to establish a desirable multiple oral dose schedule of sulfadimethoxine for adult subjects using the 0.5 gm. tablet form. The results of these studies are recorded in Table 6. It will be noted that with the 2.0 gm. daily dose (Table 6) there is a progressive rise in blood free sulfonamide level. The dose schedule of 2.0 gm. initially followed by 1.0 gm. daily produced blood levels ranging from 4.6 to 9.1 mg. per 100 ml. with no tendency to a rising level during the period of observation. An initial dose of 2.0 gm. followed by 1.5 gm. daily led to a progressive rise in the level of blood free sulfonamide. The administration of a 3.0 gm. initial dose followed by 2.0 gm. daily produced high levels consistently with a daily increase over the period of observation.

The penetration of sulfadimethoxine from the blood into the cerebrospinal fluid was measured at various intervals of from 4 to 96 hours after the onset of sulfonamide therapy in 7 patients (Table 7). Comparison of simultaneous blood and cerebrospinal fluid levels revealed a penetration of from 0 to 75% of the blood free sulfonamide level into the cerebrospinal fluid, the greater levels in general being after longer periods of sulfonamide therapy.

TABLE 7.—COMPARISON OF BLOOD AND CEREBROSPINAL FLUID LEVELS IN PATIENTS WITH NONINFLAMED MENINGES RECEIVING ORAL SULFADIMETHOXINE DAILY FOR VARIOUS PERIODS.

Patient	*Age Yrs.*	*Wt. kg.*	*Dose mg. per kg.*	*Time of Therapy (hrs.)*	*Free Sulfonamide Levels— mg. per 100 ml.*		
					Blood	*C.S.F.*	*% C.S.F. of Blood*
1. I.M.*	14	52	38	4	5.9	0.2	3
2. W.F.*	14	46	65	6	6.2	0.0	0
3. C.G.†	0.3	3.5	60	11	9.2	1.3	14
4. M.L.*	29	68	30	28	11.1	1.6	14
5. B.O.*	38	56	35	29	9.5	1.9	20
6. A.C.*	57	70	30	56	11.5	1.4	12
7. L.D.†	1	10	25	96	3.1	2.3	75

*Tablet medication
†Suspension medication

CLINICAL EVALUATION. Preliminary clinical evaluation of the tolerance and clinical efficacy of sulfadimethoxine was carried out in 369 children. The results of these studies are described in Tables 8 and 9.

In general, an effort was made to select patients (Table 8) for treatment

with sulfadimethoxine who presented a distinct clinical condition and one in which the results of treatment could be clearly evaluated. For this reason patients with otitis media, tonsillitis and pneumonia were more frequently chosen for treatment. Medication was prescribed on the basis of a dosage schedule of 1.0 gm. per M^2 initially, followed by 0.5 gm. per M^2 per day for a minimum of 6 days thereafter. In many patients treatment was continued for 10 to 25 days. As often as consistent with the physician's judgment of the therapeutic urgency of the child's condition, sulfadimethoxine was used as the sole antimicrobial agent (79%). The overall results of treatment were considered satisfactory in 86% of the patients treated as evidenced by

TABLE 8.—DIAGNOSIS AND RESULTS IN THE TREATMENT OF 369 CHILDREN USING SULFADIMETHOXINE ALONE OR IN COMBINATION WITH OTHER ANTIMICROBIAL AGENTS.

Diagnosis	*Number Patients*	*Number treated with*		*Results of treatment*	
		Sulfa. alone	*Sulfa plus**	*Satisfactory*	*Unsatisfactory*
Otitis Media	94	76	18	81	13
Bronchitis	43	42	1	42	1
Tonsillitis	47	38	9	45	2
Bronchopneumonia	34	16	18	29	5
Lobar Pneumonia	28	7	21	25	3
U.R.I. with Fever	20	19	1	19	1
Local Skin Infection	14	12	2	11	3
"Pertussis"	6	6	0	2	4
Bronchiolitis	7	6	1	5	2
Pyelitis or Cystitis	6	5	1	6	0
Gastroenteritis	5	3	2	4	1
Miscellaneous†	21	18	3	17	4
Prophylaxis‡	44	44	0	—	—
Total	369	292	77	286	39

*The other antimicrobial agent used was penicillin in all but 2 patients who received chloramphenicol.
†The miscellaneous group includes thrombophlebitis-2, acute bacterial meningitis-4, sinusitis-2, tuberculous pneumonia-1, herpangina-2, asthma-4, rubeola-1, varicella-1, acute lymphadenitis-3, and acute parotitis-1.
‡These patients were given 7 to 10 days sulfadimethoxine therapy because of contact with siblings known to have meningococcal infections.

TABLE 9.—COMPLICATIONS OCCURRING IN THE COURSE OF THE ADMINISTRATION OF SULFADIMETHOXINE TO 369 PATIENTS IN RELATION TO THE DURATION OF SULFONAMIDE THERAPY AT THE TIME THE COMPLICATION APPEARED.

Symptom or Sign	*Duration Sulfonamide Therapy (days)*			
	1–7	*8–13*	*14–21*	*22–26*
Vomiting	9	1	0	0
Rash	3	1	0	0
Urticaria	1	0	0	0
Transient neutropenia	0	0	0	1

either definite improvement or complete clearing of the disease process at the time of a follow-up clinic visit.

Complications (Table 9) in the course of sulfadimethoxine therapy were infrequent and generally not serious. Ten patients vomited, 9 within the first week of treatment. In only 2 cases was the vomiting sufficient to necessitate discontinuation of oral sulfonamide therapy. Since children frequently show vomiting as a symptom of infection this incidence is felt to be quite low. Four patients exhibited a transient rash and one other a transient urticaria. The relation of the rash to sulfadimethoxine therapy could not be established clearly since many of the childhood infectious diseases may be associated with mild skin eruptions. No child showed any desquamation or persistent skin condition. One patient receiving 1 gm. per M^2 daily developed a transient neutropenia after 24 days of treatment; it promptly disappeared when the drug was discontinued. This child was on no other medications. No significant changes were noted in the urinalyses of the patients receiving sulfadimethoxine.

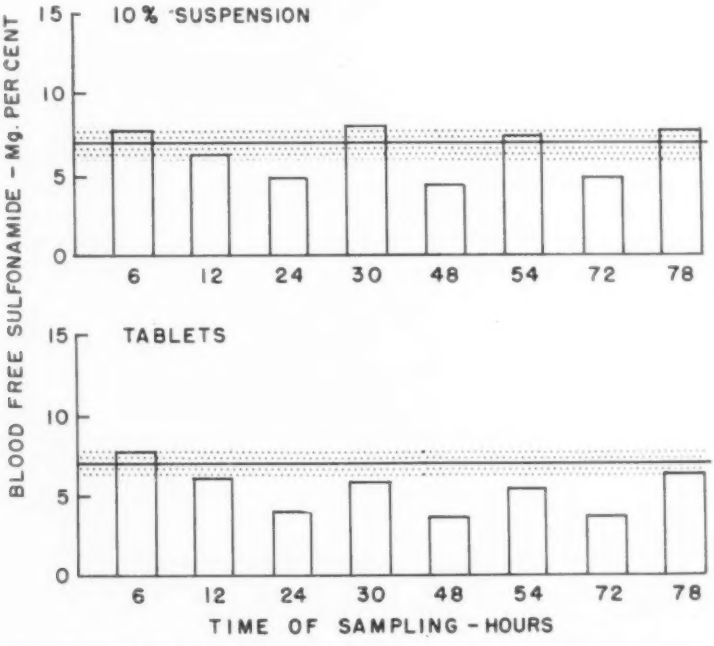

Fig. 3.—The average blood free sulfonamide levels achieved in children following the oral administration of sulfadimethoxine according to a multiple dose schedule (mean values from Table 4).

Discussion. The therapeutic use of sulfonamides has been made more convenient by the recent introduction of long acting forms. Although there is no consistent agreement concerning the ideal therapeutic level to be achieved for sulfonamide treatment, a range of 6 to 8 mg. per 100 ml. includes the recommendations of most workers (Townsend and Borgstedt[5]). Sulfadimethoxine is well absorbed by infants and children following the oral admin-

istration in either the tablet form or the 10% microcrystalline suspension as illustrated in Fig. 1. The relatively more rapid absorption of sulfonamide in patients given the suspension form may be due to its preparation as a finely divided, microcrystalline particle with a high ratio of surface area to mass.

The pattern of urinary excretion of both free and total sulfonamide following oral administration was examined in 6 children. Figure 2 is a composite graph utilizing the average values for each 12-hour period expressed as per cent of dose. The daily excretion is expressed by bar graphs and the total cumulative excretion by a line graph. It will be seen that throughout the 96-hour period of observation there is a significant quantity of sulfonamide excreted in the urine in the free or therapeutically active form. The quantity of total sulfonamide recovered from the urine suggests excellent intestinal absorption of the single oral dose of sulfadimethoxine given to each of these 6 children.

The blood sulfonamide levels in children following multiple oral doses are summarized in Fig. 3. These data suggest that a dose schedule of 0.5 gm. per M^2 per day initially, followed by 0.25 gm. per M^2 per day thereafter is usually sufficient to maintain blood free sulfonamide levels above 4 mg. per 100 ml. For serious infections a higher blood level is usually desirable and a dose schedule of 1.0 gm. per M^2 per day initially, followed by 0.5 gm. per M^2 per day should be more effective.

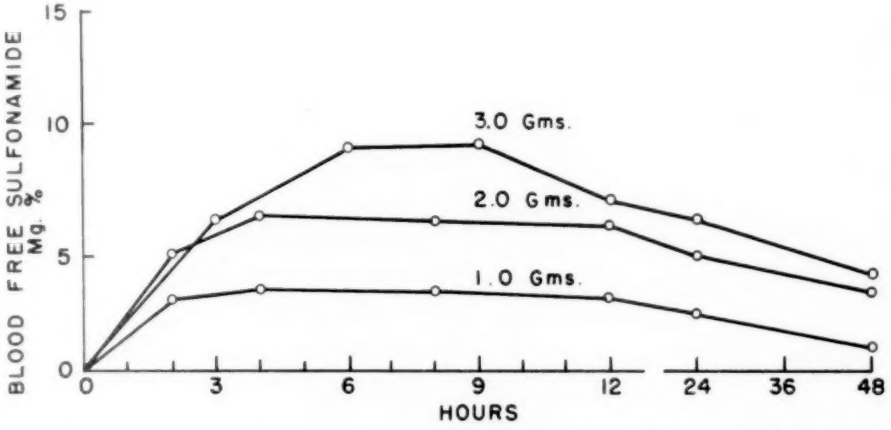

Fig. 4.—Comparison of the average blood free sulfonamide levels in adults following the oral administration of sulfadimethoxine at several dose levels and using the tablets (mean values from Table 5).

The absorption of sulfadimethoxine in the 19 adults as measured by the level of blood free sulfonamide is compared for different dose levels in Fig. 4. The pattern is similar and the levels appear comparable to the increase in dosage.

Several multiple dose schedules for the administration of sulfadimethoxine were studied in adult subjects. Figure 5 represents a summary of these studies and suggests that an initial dose of 2.0 gm. followed by 1.0 gm. daily produced a desirable and sustained level of free sulfonamide in the blood. The

other schedules studied give excessive blood free sulfonamide levels and should not be used.

Conclusions. 1. Following the ingestion of 0.5 gm. per square meter of body surface of sulfadimethoxine in suspension or tablet form, therapeutic blood levels are reached in children within 2 to 6 hours and are maintained for 8 to 9 hours. When the drug is given in a microcrystalline suspension, absorption is more rapid.

2. An initial dose of 0.5 gm. per M^2 of sulfadimethoxine followed by 0.25

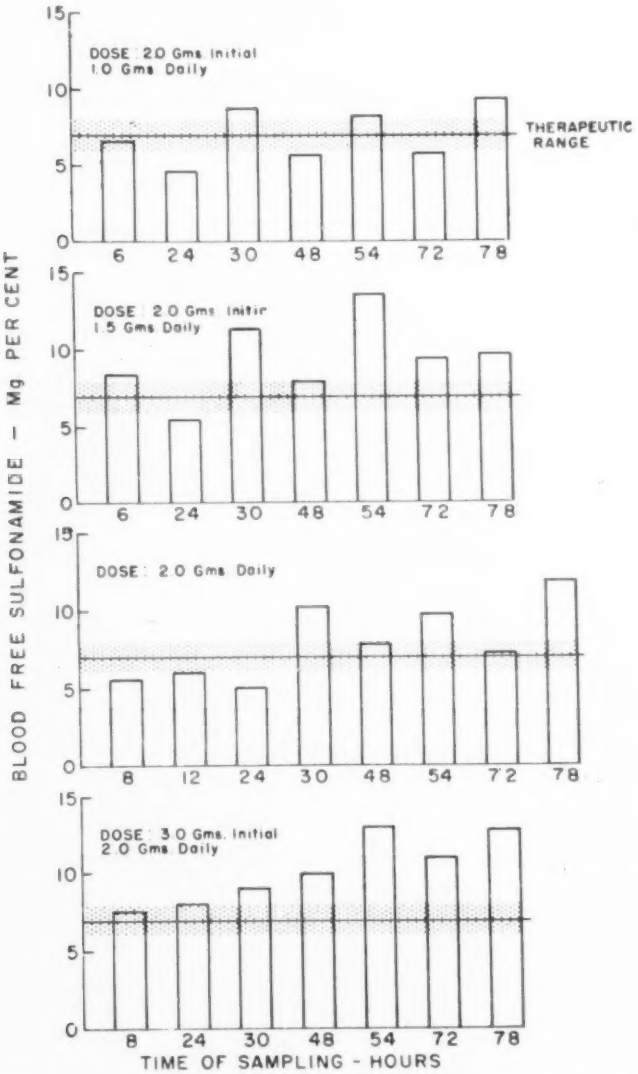

Fig. 5.—Average blood free sulfonamide levels achieved following the oral administration of sulfadimethoxine to adult subjects according to 4 multiple dose plans (mean values from Table 6).

gm. per day produces adequate blood levels in most patients.

3. In adults a single dose of 2 gm. administered in tablet form reaches levels of from 5 to 7 mg. per 100 ml. in 2 hours, and these are maintained for 24 hours.

4. When initial doses of 2.0 gm. followed by 1.0 gm. daily are administered to adults, constant levels between 5 and 10 mg. per 100 ml. are maintained.

5. Spinal fluid diffusion in patients with normal meninges is poor.

6. A large proportion of the drug is excreted in the free form, and these studies suggest almost total absorption of the drug from the intestinal tract.

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SUMARIO IN INTERLINGUA

Studios Relative al Absorption, Distribution, Excretion, e Uso de un Sulfonamido a Longe Activitate (Sulfadimethoxina) in Juveniles e in Adultos

Ha essite monstrate que sulfadimethoxina es, in animales, un efficace agente antibacterial con interessante proprietates pharmacologic. Illo es absorbite quasi quantitativemente post administration oral e persiste in le sanguine durante longe periodos de tempore, principalmente in le forma sulfonamidic. Unic doses oral produce nivellos therapeutic durante 24 a 36 horas. Le excretion occurre via le renes. In le renes solmente un micre proportion es presente in le forma acetylate. Le presente studios esseva interprendite pro determinar le absorption, distribution, penetration, e excretion de sulfadimethoxina in juveniles e in adultos. Studios clinic del acceptation e toxicitate esseva effectuate in plus que 360 juveniles. Le resultatos de iste studios suggere que sulfadimethoxina es ben absorbite (Fig. 1) e que un grande parte de illo es excernite como le libere, *i.e.* therapeuticamente active sulfonamido (Fig. 2). Un programma de multiple doses oral in juveniles como tractamento pro leve infectiones es 0,5 g per m^2 per die initialmente, sequite subsequentemente per 0,25 g. per m^2 pr die. Isto deberea resultar in nivellos de sulfonamidos libere de plus que 4 mg per 100 ml. In le caso de plus serie infectiones, un programma de doses de duo vices illos mentionate es recommendate. In adultos (Fig. 3), un programma de dosage multiple de 2 g per die, sequite per 1 g per die produceva nivellos sufficiente pro le tractamento de infectiones sever. Le diffusion de sulfadimethoxina in le liquido cerebrospinal es mal. Studios in patientes con inflammation de meninge es in progresso.

PROGRESS
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UNDER THE CHARGE OF

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THE WANING JOINT

BY M. R. JEFFREY, M.D., M.R.C.P.

(From the Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana)

As the shades of ev'ning close,
Beck'ning thee to long repose;
As life itself becomes disease, . . .
Burns

OF all the ailments by which the flesh is afflicted in the autumn of life, those arising from the failure of articular structures to endure for their proper span are amongst the most frequent. They are thus the proper concern of every physician, especially in these days when hygiene and medicine have so greatly reduced the impact of diseases which were formerly lethal in youth and middle life. The increasing numbers of people who attain a fulness of years emphasize our scanty knowledge of the mechanisms of aging in diarthrodial joints: if this review should kindle a few sparks of interest in this particular challenge, its purpose will have been well served.

PREVALENCE. Osteoarthritis* occurs widely throughout the animal kingdom, having been described in Egyptian mummies, prehistoric man, the primates and many domestic and laboratory animals. In this the disease is remarkably different from rheumatoid arthritis which is confined to *homo sapiens*.

In studying the question of prevalence the answer, as in other matters, depends to a great extent upon the question asked, any figures obtained being influenced by the diagnostic methods and the criteria employed. In clinical surveys diagnostic criteria must be chosen with care, for confusion with rheumatoid arthritis may readily oc-

*None of the names given to this process is unexceptionable, so the shortest and widely understood term "osteoarthritis" is used throughout this paper.

cur[26], and the figures obtained will, in the main, include only patients who have or have had symptoms. Radiological studies, though still subject to "observer error," are more objective, particularly when standard radiographs are used for grading purposes[67]. They reveal a much greater prevalence of the disease and detect slight deviations from the normal, which may not have given symptoms and which may never do so. Pathological studies offer the most accurate diagnosis of the nature and extent of the disease, but are obviously limited in their scope and subject to the usual hospital selection factors. Their very comprehensiveness raises acutely the question: Where does normal aging end and osteoarthritis begin?

The pathological studies of Heine[51], some of which Collins[27a] has summarized as a table, showed that naked eye evidence of cartilage deterioration begins to appear in the knee joint at an early age, the incidence jumping sharply from 9% in the third decade to 48% in the fourth, and reaching 100% in those over 80. The knee was preeminent among the major joints examined in the frequency and severity of lesions at all ages and was followed by the great toe, hip, and elbow, little difference being apparent between these three joints. Other pathological studies are in broad general agreement[9,63].

Using a standard system of grading[67], Kellgren and Lawrence[68] examined a 1 in 10 sample of the population, aged 55 to 64 in an English industrial and mining town. They found that 83% of men and 87% of women had some radiological evidence of osteoarthritis, the joints examined being the hands, feet, pelvis, knees, cervical and lumbar spine. The distal interphalangeal joints were the most often affected, followed by the first metatarsophalangeal and the knee joints. One third of the men having osteoarthritis gave a history of previous injury at the site, but spinal disc disorders and osteophytosis were considerably more common in men than involvement of the diarthrodial joints. The author's figures for the hip, knee, and great toe can be compared with those of Heine, and in the case of moderate or severe osteoarthritis agreement is close. In considering all grades of severity, however, Heine found a much greater prevalence, for whereas Kellgren and Lawrence found osteoarthritis in 35% of their subjects (average of male and female values), Heine's figure for the same group is around 90%. In large part this difference probably reflects the inability of radiology to reveal the earliest cartilage changes with certainty.

In an earlier clinical survey in the same town[69], a sample of people of all ages over 15 years were questioned about the occurrence of rheumatic pains in the preceding 5 years. Those confessing to pain, at the time of interview or previously, were examined by a physician. Symptoms due to osteoarthritis were found in 5.8% of all men and 9.4% of all women and this disorder was the largest diagnosed cause of symptoms in women and a close second to disc disorders in men. In men and women over 50, 15% and 22%, respectively, had complaints attributable to osteoarthritis; 12% of the affected men and 17% of women had lost 3 months' work or more during the preceding 5 years and osteoarthritis was, in fact the cause of the greatest loss of working time in women. Nevertheless, half of the women with symptoms had never consulted a doctor.

Clearly it would be wrong to extrapolate these figures in detail to other communities and countries. But they illustrate the order of magnitude of a

world-wide medical problem with considerable industrial implications[91]. There are perhaps some indications for moderate optimism to be derived from the figures. The presence of a clear history of trauma in one-third of the male sufferers at least points to one way of reducing the incidence of the disease. The fact that half of the women with osteoarthritis had never seen a doctor partly reflects the still too prevalent attitude that "they can't do anything about arthritis." But it also probably indicates that symptoms were often mild and that with advice about muscle rehabilitation, weight reduction and sparing the joints, a large number of patients could be enabled to complete their days with reasonable comfort and adequate mobility.

PREDISPOSING FACTORS. Although a tendency to osteoarthritis may be inherited in some cases (see below), this is essentially a local disease, the expression of a joint's inadequacy to endure the stresses inseparable from function, and each affected joint demands separate explanation. When abnormal stresses act on a normal joint or normal stresses upon an abnormal joint, osteoarthritis is likely to ensue. As Collins states, "It is exceptional to see advanced osteoarthritis in a joint without being able to discover some aggravating circumstance or condition"[27b]. Quite frequently, however, the normal use of the joint, in which no previous abnormality can be demonstrated, suffices to produce osteoarthritis. In such instances the disease tends to be relatively mild and to blend imperceptibly onto those gentle changes which lead to the gradual lessening of activity known as growing old.

Possible predisposing factors are many and mostly obvious. Collins classifies them as those that injure cartilage primarily or those that impose abnormal mechanical conditions, increasing the stresses on joints[27b]. In the first class are such conditions as direct trauma, epiphyseal abnormalities, inflammatory arthritis and metabolic disorders like gout or ochronosis; in the second are deformities from bone disease or malunited fracture, malalignment due to postural defects and abnormal stresses from obesity or excessive use.

In the hip an important predisposing cause, apart from the obvious ones, is congenital dysplasia[78]. In one series this was responsible for 26 out of 124 osteoarthritic hips requiring surgery[80] The condition is recognized in AP radiographs with the hips in neutral position by comparing two lines: (a) A line caudad, at right angles to a line joining the centers of the femoral heads, (2) A line from the center of the femoral head to the outer border of the roof of the acetabulum.

In the normal hip these lines form an angle of 25° or more; below 20° dysplasia is definitely present, provided flexion or rotation of the hip are excluded[80]. Since this condition was responsible for one-fifth of hip operations for osteoarthritis and the average age at operation was 31 years, its early recognition and the seeking of an orthopedic opinion in suspected cases are of great importance.

The knee is subject to many malign influences. In a population survey a clear relation was shown between obesity and the occurrence of the more severe grades of osteoarthritis[68]. There is a higher incidence of osteoarthritic knees in women, simply because more of them are too fat.

The roles played by diet and obesity have been extensively studied in the mouse. High fat diets accelerate aging and enhance the development and severity of osteoarthritis in mice[107]. The effect of fat is particularly notable in grossly obese animals[106] but depends

on other factors besides increased weight, since equicaloric carbohydrate diets produce a much lesser increase in osteoarthritis[114,117]. Furthermore, in mice receiving a high fat diet, joint changes may precede marked obesity[118]. The deleterious effect of a high fat diet is less in strains with relatively high metabolic rates[119], in older animals[113] and in the female sex[108], but is enhanced in the spayed young mouse[120]. On the other hand, restriction of total caloric intake or the feeding of a high protein diet[107,109,114] reduces the incidence of osteoarthritis, probably mainly by retarding the normal development of articular tissues.

How far these results are applicable to man is not known, though the general ill effects of obesity and the benefits of calorie restriction need not be doubted. The physician might well be justified in referring dogmatically to the aging effect of fat when trying to insure the cooperation of his female patients in dieting.

During the routine examination of students, Parnell[95] found a surprisingly high incidence (18.3%) of apparent minor knee effusion. The proportion rose steadily with age and it was suggested that the effusions represented an early manifestation of osteoarthritis. Unfortunately no effusion was confirmed by aspiration, the diagnosis being based upon transmitted bulging beneath the patellar tendon. If this finding were confirmed, the prospects of preventing more serious damage by controlling weight and advising against unsuitable occupations or sports might be considerable.

It was also noted that students with small bones and relatively heavy weight had effusions twice as often as those with large bones and a low weight. This agrees with Seltzer's suggestion[102] that osteoarthritis of weight-bearing joints occurs in people of a particular anthropometric type who have a large body and relatively slender limbs, ill endowed to bear their burden. Clearly, people of such a build might be expected to develop osteoarthritis relatively often for purely mechanical reasons and the postulation of a diathesis in addition seems at first sight unnecessary. There is, however, evidence in its favor from studies of heredity and from population surveys.

Stecher[128,129] has shown that Heberden's nodes may be of two types, namely, idiopathic or traumatic. The idiopathic nodes show a marked predilection for women (about 10 to 1) and the female relatives of patients are often affected. The condition seems to be inherited as a single autosomal characteristic which is dominant in women and recessive in men. In a population survey, using radiological evidence for diagnosis, a highly significant correlation was found between Heberden's nodes and the presence of widespread osteoarthritis, affecting the other small joints of the hands, the feet, knees, and interfacetal joints of lumbar and cervical spine. Also, obese men had a high incidence of Heberden's nodes in addition to osteoarthritis of the weight-bearing joints. It is therefore postulated that the hereditary factor transmits a susceptibility to osteoarthritis in general rather than to Heberden's nodes in particular[68]. It is well known that among laboratory animals there are marked strain differences of a hereditary nature in the incidence of osteoarthritis[107,119,126]. It may well be that Seltzer's anthropometric type is linked to a hereditary susceptibility to osteoarthritis.

The predisposing role of occupation depends essentially on the degree of trauma involved. Thus coal miners, who are subject to knee and back injuries and may have to work in cramped conditions, are unduly liable to osteo-

arthritis of the knees and spine and to intervertebral disc lesions[68]. Predisposing injuries do not need to be major; the prolonged strenuous use and repeated trivial injuries of professional sports players or of workers with pneumatic drills can be just as damaging. Men are much more liable to trauma both in their jobs and hobbies than women, which probably explains the different patterns of joint involvement in the sexes. Except to Heberden's nodes, sex in itself is not an important predisposing factor. Liability to trauma may also explain apparent racial differences in incidence[65].

The role of endocrine factors is probably small in human osteoarthritis. Stecher[131] noted that, in 44 out of 99 women with Heberden's nodes, the onset of the nodes and the ending of menstruation occurred within 3 years of each other, a proportion greater than would be expected by chance. This effect was not observed by Kellgren and Moore[70] and, in any case, the tendency of patients to attribute illness of all kinds to "the change" makes its significance doubtful. The pituitary can probably be exonerated, for the joint disease occurring in acromegaly is not the same as osteoarthritis[66] and the urinary output of gonadotrophins, corticoids, 17-ketosteroids, and estrogens is not abnormal in osteoarthritic patients[99]. Attempts to establish the significance of endocrine abnormality at the time of the menopause or later seem foredoomed to failure since pathological studies have shown that the disease commonly first appears 20 or 30 years earlier.

The effects of endocrine alterations upon the development of osteoarthritis in various strains of mice are somewhat complex. It appears that factors which retard the normal development of epiphysis and bone in young mice will reduce the incidence of osteoarthritis; among these factors are ovariectomy[116], ACTH[111], orchidectomy[115,121], and thyroidectomy[112]. The implanting of adrenal grafts in spayed animals still further reduces the incidence of osteoarthritis[115]. Thyroidectomy, however, produces another kind of joint disease, characterized by mucoid and hyaline change of menisci, ligaments and synovial membrane with retarded ossification. The severity of this "athyroid" disease is greatly influenced by the natural level of thyroid activity of the mice examined, strains with high levels being much more affected than those with low levels[112].

The implantation of anterior pituitary grafts increases the development of osteoarthritis in normal and spayed mice, but this effect is antagonized by simultaneous adrenal or ovarian grafts in young males[115]. Osteoarthritis is accelerated, too, curiously enough, by thyroidectomy in the mature animal[116].

Thus, the situation is complex, and the existence of marked differences between species[127] and between strains of the same species[107,119,126] makes the direct application of experimental findings to man hazardous in the present state of knowledge.

ARTICULAR CARTILAGE. *Structure.* Hyaline cartilage is composed of three elements; cells, matrix and fibers. Only brief comments can be made here; for details the reader is referred to a series of papers on the basic structure of connective tissues[140] and a succinct review[19].

Cartilage cells tend to be arranged in rows perpendicular to the surface, the deep cells often existing in small clumps[27c]. Although the oxygen consumption of cartilage is negligible, the cells are by no means inert, having a glycolytic activity per cell comparable to that of other tissues[18]. In young rats sulfate labeled with radiosulfur and injected intraperitoneally was detec-

table in the vicinity of the walls of young cartilage cells and in the intercellular substance 19 hours after administration, reaching a maximum at 48 hours[36]. Labeled sulfate is retained in the cells of epiphyseal cartilage even more rapidly[7,29] and after 4 hours can only be removed with difficulty. Seven hours after the intravenous injection of S_{35} sulfate the articular cartilage of adult rabbits yields strong autoradiographs. Radioactivity is at first confined to the chondrocytes and the average intensity of blackening in the various parts of the cartilage is proportional to the number of cells per unit area[21]. Activity later spreads into the ground substance, thus confirming previous ideas that chondrocytes are responsible for the synthesis of the mucoids of the matrix and emphasizing the important but poorly understood activity of these cells.

The ground substance of the connective tissues has been widely investigated in recent years[19,140]. The characteristic constituents are mucopolysaccharides or mucins, which are highly polymerized, fundamentally carbohydrate substances combined with protein to form a gel whose chemical complexity is beyond the scope of this review. Chondroitin sulfate is the characteristic carbohydrate constituent of hyaline cartilage and is estimated chemically as the amino sugar or hexosamine liberated when it is broken down. The mucopolysaccharides impart the characteristic metachromasia with toluidine blue which is more intense (and the degree of polymerisation presumably greater) in the deeper zones than superficially[40]. The mucopolysaccharides are largely responsible for the resilience of hyaline cartilage, being particularly abundant in areas of special stress. The ratio of chondroitin sulfate to collagen is greater in cartilage from the weight-bearing area of the knee than in the nonweight bearing cartilage from the periphery. In the shoulder, a non-weight bearing joint, the ratio of chondroitin to collagen is similar to that in the peripheral cartilage of the knee[85].

There is a general tendency for the mucin content of connective tissues to decrease with advancing years. The process of growth is associated with a decrease in the ratio of hexosamine to collagen in human skin and this decrease continues throughout life, so that at death the ratio is approximately half that obtaining at birth[124,125]. In human costal and femoral cartilage the content of chondroitin sulfate decreases with advancing age[82,132] and the viscosity and hexosamine content of synovial fluid also decrease[46,60]. It has been suggested that a decrease of the gel to fiber ratio is a characteristic feature of the aging of connective tissues[125] which may be accelerated by chronic illness[124] or by corticosteroid substances[125].

The fibers in cartilage may be revealed by connective tissue stains and are composed of collagen with its typical appearance under the electron microscope. Little is known directly of the metabolism of cartilage fibers, but collagen fibers from the tendons of young animals show active anabolism, glycine labeled with C_{14} being rapidly incorporated. Once formed, collagen seems to be almost inert and the specific activity remains constant at least for several weeks. In older animals little incorporation of C_{14} glycine occurs[93].

The fibers and matrix of cartilage are intimately connected. On examining an acetone dried powder of bovine hyaline nasal cartilage, Partridge[96] found that one-third of its mucoid could be extracted by cold distilled water. After heating the powder to 60 to 70° C. (the shrink temperature

of collagen, at which its physical properties alter profoundly) a further one-third of the original mucoid content could be extracted. The last third was only extractable by agents which depolymerized the chondroitin. Jackson[58] found that treatment of calf tendon with hyaluronidase markedly increased its solubility in dilute acetic acid and reduced its shrink temperature. It appears that some mucopolysaccharide is intimately associated with collagen fibrils and is essential for their stability[19].

The possibility of degradation of collagen with increasing age has been raised[123] and Verzár has shown that the tail tendon of old rats shrinks at a lower temperature than that of the young[142]. However, this could be a function of diminished mucoid, for the prominent change with increasing age shown by electron microscope examination of human dermis consists of loss of amorphous material around the collagen fibers rather than alteration of the fibers themselves[61].

The fibers of cartilage have a characteristic distribution[10]. Superficially, they lie parallel to the surface in a feltwork; slightly deeper, they turn away from the surface and form a transitional criss-crossing zone. Deeper still, they run perpendicular to the surface and pass into the zone of calcified cartilage and reach the underlying bone. This pattern has been confirmed by electron microscopy[79], which showed that in middle age the fibers were unorientated in the outer two-thirds of normal cartilage except in the most superficial tangential layer. Orientation perpendicular to the joint's surface was only seen normally in the inner one-third of the cartilage.

Nutrition. Normal hyaline cartilage is completely lacking in blood vessels, and in keeping with its ischemic state, its metabolism is almost entirely glycolytic[18]. As long as it is bathed by synovial fluid, cartilage remains alive and apparently normal[75], even when isolated from its previous bony attachment, as in osteochondritis dissecans, or after surgery[41]. The movement of fluid into and out of cartilage is presumably helped by its elasticity and sponge-like characteristics. Under direct pressure cartilage becomes thinner, at first rapidly, and thereafter much more slowly; with a continuous moderate pressure it continues to yield slowly and equilibrium is not established within 30 minutes. During this compression beads of fluid appear around the point of pressure and the deformity of the cartilage is greater, the smaller the area over which pressure is applied. After short-lived pressures, even if large, elastic recoil may be complete almost instantly, but if pressure has been repeated or sustained, the immediate recoil is only partial and full recovery takes place much more slowly[52].

Bywaters[18] calculated that articular cartilage of normal thickness could be supplied with its nutritive needs by diffusion, though this might not be adequate for thickened cartilage or when synovial permeability was impaired. With the additional pumping effects of normal joint movement, it would appear that nutrition can be more than adequately ensured by synovial fluid.

Evidence in favor of an additional source of nutrition from subchondral bone has been presented by several workers. In rabbits a small proportion of the basal aspect of the calcified layer of articular cartilage is in contact with the medullary cavity of bone[55] and capillaries are present in these areas. When starch granules were injected into the marrow cavity of the epiphysis of rabbits and the adjacent joint was then repeatedly compressed to simulate running, starch could subse-

quently be demonstrated within the articular cartilage. It was also found within the cartilage after intravenous injection, but not after injection into the joint cavity, even when the joint had been "exercised"[57].

Further evidence was given by Ekholm[38], who injected radiogold chloride intravenously. After one hour the femoral cartilage of the normal animal contained significantly more radioactivity than the tibial. When the injection was given into the tibial epiphysis, however, the tibial cartilage contained more activity than the femoral. This was probably not a direct pressure effect, forcing fluid into abnormal channels, as the injection volume was small and was given under low pressure over a period of several minutes; nor was it likely to be an effect of pumping the injection into the circulus vasculosus, for this is only in contact with the periphery of the cartilage, whereas specimens were taken from the central part.

These experiments also showed that the activity of the synovial fluid after intravenous injection of radio-gold was ten times greater than that of cartilage, and that after repeated passive movements of one knee higher concentrations were attained in the cartilage and synovial fluid of that joint than on the unexercised side. When one knee was repeatedly irrigated with bovine synovial fluid during the hour after intravenous injection of radio-gold, less activity appeared in the cartilage than in the control knee[38].

These findings support the nutritive role of synovial fluid and demonstrate the increased transfer of fluid into cartilage resulting from joint movement. There is little doubt also that in the rabbit fluid can pass from medullary cavity into cartilage[37,38,57]. The extent of this phenomenon in man is uncertain but probably small. Landells[75] found only scanty vessels in the zone of calcified cartilage, "too few to have much responsibility for cartilage nutrition." Hyaline cartilage is, in fact, virtually an obligatory anaerobe; penetration of capillaries through an incomplete fracture of subchondral bone leads to removal of mucopolysaccharide matrix around the advancing loops, followed by aggregation and approximation of collagen fibers and death of cartilage cells[75]. Hyaline cartilage and a blood supply are incompatible[137].

It seems reasonable to conclude that although the calcified zone of cartilage may derive some nutrition from medullary blood vessels, in normal circumstances hyaline cartilage in man is nourished for practical purposes by synovial fluid, whose ebb and flow is aided by the alternate compression and relaxation of normal joint movement.

PATHOLOGY. The resilient milky-white articular cartilage of adolescence does not long retain its pristine smoothness. Senile changes begin to appear in the second and third decades in the knee joint[51]. Collins, whose book should be consulted for an admirable description of the pathology, states that, "After the age of thirty, an entirely normal smooth weight-bearing cartilage is unusual"[27d].

In general, the earliest changes occur in the articular cartilage at the sites of greatest stress or pressure; for example, in the patellofemoral compartment of the knee[63]. "An erster Stelle steht hier die Patella"[51]. At these sites the cartilage becomes first a little frayed and blistered, superficial flakes are lost and cracks appear. With further use more cartilage is worn away and the fissures deepen into furrows. In joints whose motion is almost confined to one plane, ridges and furrows in opposed surfaces may adapt to fit each other. Meanwhile, the immediate subchondral bone undergoes remodeling and thickening, while at the circumference of the car-

tilage proliferation of bone and cartilage is seen. Finally, weight-bearing cartilage is wholly worn away, exposing dense, eburnated subchondral bone, deep to which there may be thickening of some trabeculae, atrophy and collapse of others. There are now obvious marginal osteophytes and the joint capsule may show some fibrosis and the synovial membrane be a little congested. In time, the eburnated surfaces may develop mutually adapted ridges and furrows, for the bone ends, though dense, are poorly able to withstand the inevitable trauma of use[80] and may become extensively remodeled and distorted.

Histologically, the first disturbance of the normal orderly arrangement of cells and fibers occurs at the surface of the cartilage. There is superficial loss of cartilage substance in the plane of the tangential fibers so that fraying occurs and flat flakes may be lost from the surface. With further loss of tissue, fissures spread more deeply into the cartilage substance, following the plane of the fibers, and the characteristic appearance of fibrillation is produced by these perpendicular cracks. At this stage the electron microscope reveals loss of matrix and a realignment of fibers, which now run a parallel course perpendicular to the joint surface through almost the whole thickness of the cartilage[79]. Thus, perpendicular fissures can develop after a much slighter loss of superficial cartilage than would be possible with the normal arrangement of fibers.

As the superficial layer is worn away, the cells immediately beneath it die and matrix is lost. In the deeper zone, the cells become swollen, clumped together, and metachromatic staining of the matrix around them is diminished, indicating depolymerization of mucopolysaccharides[75]. In time these cells, too, die as the cartilage nearer to them is worn away. Perpendicular cracks in the cartilage are usually halted at the surface of the zone of provisional calcification. This surface, which shows as a characteristic wavy line in stained sections, has been christened "the tidemark"[40], and also serves to limit the spread of vertical cracks due to direct trauma. However, cracks may run along it, parallel to the joint surface[75], resulting in the detachment of whole thickness fragments of cartilage.

CLINICAL PATHOLOGY. There is no specific test for osteoarthritis and the usual blood examinations are normal, thus only helping to exclude other diseases. The sedimentation rate in local osteoarthritis is normal, but may be mildly accelerated when a joint is secondarily inflamed by trauma; it is not uncommonly raised in patients with generalized osteoarthritis, and 8 out of 112 such patients gave a value greater than 40 mm. in one hour[70]. The serum concentrations of minerals and electrolytes and of proteins and polysaccharides are normal[103]. The agglutination tests for rheumatoid arthritis show only the occasional low titer false positive results found in the general population.

Effusion into major joints is not uncommon; the characteristics of the fluid in this and other diseases have been described in a classical monograph[100]. The fluid has a normal or increased mucin content, yielding a dense clump on addition of acetic acid, and the leukocyte count rarely exceeds 6,000 per c.mm., with mononuclear cells predominating. The globulin content, which is very low in normal fluids, may increase considerably so that there is a moderate increase of total protein concentration and the proportions of the proteins approach those of normal serum[101]. The sulfate content of synovial fluid is higher than that of serum and this has been taken to indicate increased breakdown of the chondroitin sulfate of articular cartilage[25].

The relative viscosity is within the

normal range, though the average may be lower than normal[12,60]. Anomalous or non-Newtonian viscosity[94] (a thoughtful provision of nature whereby viscosity is high at low rates of joint movement and low during rapid motion) is preserved[60]. This remarkable property of the fluid is due to its hyaluronic acid content, as is the marked resistance of a thin film of fluid to compression. Viscosity is much reduced by hyaluronidase, anomalous viscosity being particularly affected[94]. Viscosity also decreases with advancing age[60]. Calculation from the figures of Hamerman and Schuster[46] shows a highly significant negative correlation between age and the hyaluronate content of synovial fluid (estimated as hexosamine), derived from cases of sudden death. Comparison of the fluids from the knee joints of patients with osteoarthritis and rheumatoid arthritis led Fletcher[42] to conclude that their different viscosities were simply the result of different concentrations of hyaluronic acid. It would seem that depolymerization of mucin is less important in reducing synovial fluid viscosity than has been thought and that simple reduction in concentration is very often responsible.

Hemarthrosis may occur infrequently without notable trauma[147] and may be diagnosed and treated by aspiration of the joint.

PATHOGENESIS OF VARIOUS FEATURES. *Cartilage Change.* It is generally agreed that alteration of the hyaline cartilage is a fundamental feature of osteoarthritis. There are probably several factors involved in this decline, their importance varying from joint to joint.

1) NUTRITION. It has long been alleged that ischemia, in association with atherosclerosis, is an important factor and this opinion is still held[141]. Osteoarthritis and atherosclerosis may certainly be found in the same patient, but probably no more frequently than can be explained by the chance coincidence of two common diseases. Heine[51] found no relation between osteoarthritis of the large joints and atherosclerosis of the major arteries and Collins confirms this[27e]. The angiographic demonstration of diminished arterial filling in the hands of a few patients with Heberden's nodes[77] cannot be accepted as valid evidence in the absence of any controls.

A number of observations strongly suggest that ischemia is not a causative factor. Trueta and Harrison[138] showed by injecting the blood vessels of the normal femoral head that there is an impressive absence of decreasing patency of the vascular tree with advancing age. In the osteoarthritic hip there is, in fact, an increase in the vascularity of the femoral head[48] with venous engorgement and sinusoid formation. In the skeleton of a gorilla which had marked osteoarthritis of the left knee, many small holes, apparently for the passage of blood vessels, were present in the articular surface of the more severely affected lateral femoral and tibial condyles[130]. The presence of increased blood flow in osteoarthritic joints is also indicated by the facts that these joints have a higher intra-articular temperature[54] and exhibit a higher rate of clearance of radiosodium after intra-articular injection than normal joints[31]. The clearance of radiosodium from normal joints is accelerated by intra-arterial priscoline, but the higher rate from osteoarthritic joints is unaffected, indicating that vasodilatation is already present in the synovial vessels[31].

It has already been pointed out that a direct blood supply is incompatible with the continued existence of hyaline cartilage, which is nourished essentially by synovial fluid, perhaps with a small contribution of tissue fluid

through the calcified zone from subchondral capillaries. The possibility of decreased synovial fluid production or turnover, resulting from sclerosis of synovial or subchondral vessels and causing impaired cartilage nutrition, would seem to be ruled out by the studies just quoted. The frequency with which early changes are found in the third decade[51] is inconsistent with causation by atherosclerosis and the location of the earliest change at the cartilage surface, where synovial fluid is most accessible, also argues against an impairment of nutrition.

2) Toxic Effects. The damaging effects upon cartilage of uratic deposits and of homogentisic acid derivatives are well known. Although "toxins" from the intestine or septic foci are out of fashion, the role of more subtle noxious agents perhaps merits less summary dismissal. It has been shown that papain will cause rapid and widespread removal of cartilage matrix when injected into rabbits, producing collapse of the ears and other phenomena[134]. The enzyme acts by reducing the mucoprotein and chondroitin sulfate content of the cartilage[139] and appears to be locally activated in the cartilage or its vicinity[87]. Such an enzymic action might perhaps better be invoked to explain the rare human disease known as chronic atrophic polychondritis[6], in which a slow dissolution of cartilage occurs. The normal serum enzyme plasmin is capable of destroying cartilage when activated by the kinases of common pathogenic organisms and by a factor present in cartilage itself[72]. The early onset of cartilage degeneration, shown by pathological studies, at least raises the possibility that an unwanted activation or perversion of action of a normal body constituent might play some part in initiating the downfall of cartilage. The ability of influenza virus to attach to mucopolysaccharides[17], and the frequent infections of childhood are thought-provoking in this connection: likewise the vigorous use and repeated minor injury of joints in youth.

3) Wear and Tear. The use of joints is probably a *sine qua non* for the development of osteoarthritis. "A disused joint never gets osteoarthritis, a misused one often does"[27f]. The sites at which the earliest changes appear and the most severe ulceration occurs later are those subjected to the greatest stress.

In the knee, the back of the patella is repeatedly compressed against the femur in walking as the quadriceps resists the flexing effect of body weight. At the start of extension from extreme flexion, as in "knees bend," the patella is ground against the under aspect of the femoral condyles with a great force which acts through a very small area of patellar surface, owing to incongruity of this surface with the femoral condyles[52]. The early onset and severity of osteoarthritis in the patellofemoral joint is, thus, not surprising[9,51,63]. The tibial cartilage tends to be protected from damage where it is covered by menisci[63]. Damaged cartilage becomes soft and less resilient; thus it more readily suffers further damage and a vicious circle ensues. Neither in disease nor after experimental damage does hyaline cartilage show any effective capacity to replace itself[21,75]. Chondrocytes remote from the site of experimental damage may proliferate and take up S_{35} sulfate more readily but near the lesion, where repair is most needed, they degenerate and lose the power to take up sulfate[21].

Comparative pathology supports the important role of weight-bearing in osteoarthritis. The bulky mammals support their weight in considerable degree upon their forelegs and suffer to a relatively large extent from osteoarthritis in those legs. Rats

and mice, which use their hind legs a good deal for standing, have a corresponding excess of osteoarthritis in the hind limb joints[122]. In man, the excess of standing and of hind limb osteoarthritis is, of course, even greater.

Experimentally, attrition of cartilage by repeated movements of the ankle joints of recently sacrificed rabbits is slow, but is considerably aggravated by movements associated with joint compression, or when synovial fluid viscosity has been reduced by hyaluronidase[4]. If cartilage is damaged chemically in animals, osteoarthritis only occurs in limbs which are used and is prevented by nerve sections which cause paralysis, the cartilage of the paralysed limb simply undergoing fibrous replacement[16].

In patients who have had strokes, Heberden's nodes have been observed to develop only on the unparalyzed side[148]. However, the role of use and trauma in producing idiopathic nodes is doubtful, for the distal interphalangeal joints would not appear to suffer any greater stress or trauma than the metacarpophalangeal joints which are much less affected by osteoarthritis.

In distinction from the knee, cartilage change in the hip may begin in the non-weight bearing area[48], though by the time symptoms are caused, the brunt of destruction is in the pressure area[89]. Because the articular surface of the acetabulum is shaped like an inverted "U," the inferior and medial cartilage of the femoral head is in contact only with fat for most of its existence and this area of cartilage often shows the earliest degeneration[48]. As noted previously, the compression and relaxation of normal joint movement is probably necessary for cartilage nutrition. It has been shown that, if the patellae of rabbits are dislocated medially and the animals allowed to run freely, the femoral cartilage, with which the patella previously articulated, gradually becomes atrophic[8]. It may be that a similar disuse atrophy affects the uncompressed cartilage of the femoral head and this would support those who prefer the term degenerative joint disease for this condition. The position is complicated, however, for the early change in the non-weight bearing cartilage is seen most often in femoral heads with well developed trabecular "pressure cones" (see bone changes) and least in heads with diffuse trabecular architecture[48]. The prominent pressure cones suggest a localized transmission of excessive force, due perhaps to a minor incongruity or maldistribution of stresses within the joint. Whereas in a femoral head with normal trabecular architecture, weight bearing presumably exerts some diffused stress, and therefore compression, on the cartilage of the non-weight bearing area, the concentration of weight bearing stress through a very restricted area of bone might deprive the non-weight bearing cartilage of any compression and, thus, impair its nutrition. The wear and tear destruction of the weight bearing cartilage and the degenerative change in non-weight bearing cartilage could thus be envisaged as parallel phenomena having a common origin in abnormal intra-articular stress distribution, aggravated in the case of the inferomedial cartilage by its peculiar anatomical situation.

4) Alterations in Constituents of Cartilage. The importance of the mucoprotein matrix in determining the physical properties of cartilage has already been mentioned. The progressive loss of matrix with increasing age cannot but result in softening and decreased resilience of cartilage, thus facilitating its destruction. It is difficult to believe this is not an important factor predisposing to osteoarthritis in

middle and later life, though its effect may be reduced by the lessened activities of old age. To what extent matrix loss plays any part in the onset of changes in young adults is unknown. The hexosamine content of femoral articular cartilage decreases markedly during youth; by the end of the third decade the content is only about 40% of that present at birth[82]. This decrease during the most active period of life is, to say the least, unfortunate and may be suspected of laying the seeds of future trouble.

It is postulated that alteration of collagen fibers is characteristic of aging[123], but the significance of this in osteoarthritis is doubtful. The fibers of osteoarthritic cartilage are certainly aligned somewhat differently, but individual fibers appear essentially unaltered[22,79]. In general, collagen fibers are more susceptible to degradation by simple agents in youth than in adult life[62] though Verzár[142] found that the tail tendon from old rats contracted at a lower temperature than did the tendon of young rats. It is possible that the surface temperature of cartilage may at times be raised by movement or by general fever to an extent which, over a long period, might lead to fiber degeneration. The changes in cartilage temperature during motion are not known, but the relatively normal appearance of individual fibers on electron microscopy of osteoarthritic cartilage argues against an important role for this mechanism.

Marginal Outgrowths. These exostoses, so characteristic of osteoarthritis in radiographs, are of two types which differ in form and origin and are often confused. One type, to which some would restrict the term "osteophyte," consists of ossification spreading into the attachment of ligament or tendon to periosteum or bone cortex, and occurs at sites of chronic strain[11]. This type is found in the ligaments of the vertebral column (and is usually described as "osteoarthritic lipping"), in the long plantar ligament, on the tibial spines and elsewhere. These osteophytes grow only within the confines of their parent tendon or ligament and, because of their origin in tension, tend to grow together from opposite sides of a joint; as in the spine, for example. The idiopathic type of Heberden's node is a tendon osteophyte, not necessarily associated with any cartilage degeneration in the terminal interphalangeal joint[27g]. The overlying soft tissues may be swollen by a cystic mass which contains only hyaluronic acid[59]; its relation to the bony outgrowth is uncertain.

The other type, here called the marginal excrescence, is usually considered a relatively late manifestation of osteoarthritis, indicating the presence of advanced cartilage destruction in the central parts of the joint[27h], but this is not invariable[51]. The excrescences originate in the penetration of capillaries into the peripheral cartilage, transforming it into cancellous bone surmounted by fibrocartilage but not by hyaline cartilage. At first low and verrucose, they tend to continue the line of the joint's surface as they enlarge and are molded by the pressures of joint motion and of adjacent muscles and ligaments. This effect is obvious, for example, in the excrescences arising from the upper anterior border of the femoral condyles which grow directly caudad beneath the powerful quadriceps. These outgrowths take origin at the junction of articular cartilage with periosteum and bone, a highly vascular site which marks the junction of tissues derived from skeletal blastema and the outlying mesenchyme[27i]. This tissue appears to proliferate readily; rheumatoid pannus starts at this site.

The excrescences on adjacent joint surfaces tend to grow apart rather than together, so that tension in joint capsule or ligaments seems an unlikely cause for the proliferation. Invasion of the calcified zone of cartilage with its transformation into bone is characteristic of osteoarthritic joints[48,51] and this process, occurring exuberantly at the edges of joint surfaces, where pressure from opposing surfaces is absent, may be the immediate mechanism producing outgrowths. The reason for this invasion remains unsettled. The presence of outgrowths as early as the second decade[51] suggests it is a phenomenon parallel to, rather than dependent upon, cartilage destruction. The injuries and stresses which initiate degradation in cartilage appear to excite proliferation in the highly vascular tissues at the articular margin.

Bone Changes. The capillary proliferation and advance of ossification which form the excrescence peripherally also occur centrally, deep to degraded cartilage. The process is less exuberant, however, probably because of the direct pressure exerted upon capillaries by weight bearing or other stresses; it leads at first to the thickening of subchondral bone, which is a characteristic radiological feature, and later to the dense eburnated bone found when cartilage has wholly disappeared. At the same time, the underlying bone becomes remodeled so that trabeculae in the direct line of stress transmission become thickened and others may be absorbed.

In the hip joint, which has been the subject of elegant studies by Trueta and colleagues, the remodeling results in the formation of a "pressure cone" in the femoral head, the base of the cone being at the weight bearing area on the upper surface of the head and its apex pointing down to the femoral shaft[48]. The development of this cone of trabecular sclerosis and the liability of the human hip to osteoarthritis have been linked to the adoption of the upright stance and to man's unique ability to lock the hip and knee in full extension[137]. During standing, weight is transmitted through a constant, very localized area of the femoral head, and the area affected during walking is not very much greater. This region corresponds to the base of the pressure cone and is the site of the most advanced cartilage destruction in osteoarthritis. That this cone does correspond with the region responsible for weight transmission was confirmed by Fessler[41], who studied stress trajectories in a plastic model of a flat coronal section of a hip joint and showed a remarkable similarity between the lines of stress and the architecture of the femoral head.

Further developments in the pressure cone and acetabular roof, and less commonly in sclerosed cancellous bone adjacent to other osteoarthritic joints, lead to bone collapse and the formation of "cysts." Deprived of the resilient protection of articular cartilage the femoral head is exposed to the full stresses and shocks of weight bearing, which may lead to fracture of trabeculae weakened by remodeling and perhaps by the hypervascularity which is present[48]. In the center of the cone, bone resorption occurs and trabeculae are replaced by loose connective tissue or by mucinous fluid, occupying a roughly spherical space and leading to the radiological appearance of a cyst.

The exact genesis of these cysts is uncertain. Slow ischemic atrophy resulting from capillary closure by the high pressure in the very localized weight transmitting segment of bone may play a part[137], but probably more important is the role of fracture of the overlying cortical bone, following minor trauma and allowing intra-

articular pressure to be transmitted within the medullary cavity. The cysts are found deep to exposed, but not eburnated bone[74], which, lacking the protection of cartilage and weakened by previous invasion of blood vessels through it into the calcified cartilage, might be expected to crack easily; careful examination does, in fact, usually reveal a communication with the joint cavity[74,98,137]. The smoothly rounded contours of the established cysts are certainly best explained by the transmission of hydrostatic pressure. The presence at times of a glairy fluid, lacking cholesterol, fibrin or blood, and the demonstration of fragments of hyaline cartilage, which is not metaplastic, lying in their walls point strongly to the intrusion of joint contents within the bone[74]. The marked vascularity and subsequent dense sclerosis of bone in the wall of the cysts[48,137] are presumably "reactive phenomena" and are reminiscent of the changes around a Brodie's abscess.

The Genesis of Symptoms. The discomfort, difficulty in movement and feeling of instability associated with the advanced disease are readily understandable on the basis of capsular fibrosis, irregularity of joint surfaces with marginal excrescences and perhaps detached cartilage fragments, and abnormality of proprioceptive impulses resulting from the altered relations and scarring of joint constituents and from weakening of muscles. The characteristic stiffness which follows immobility and the occasional severe deep pain are less immediately explicable.

In contrast to the stiffness of rheumatoid arthritis, which is probably due to inflammatory edema and infiltration into joint tissues and is but slowly eased by movement, the stiffness of osteoarthritis is abolished by only a few moments of gentle limbering up, in a way which suggests the breaking down of frictional forces rather than the slow absorption of inflammatory exudate. The general tendency for mucoprotein in connective tissues to decrease with advancing years has already been mentioned and in joint cartilage at least rest leads to thinning, whereas exercise produces swelling[57]; in tendon repeated use leads to increased mucopolysaccharide content[149]. If these changes are representative of all connective tissues the stiffness of old age and osteoarthritis may be envisaged as due to the formation of abnormal linkages, perhaps between fibrous proteins, linkages prevented in younger and more active life by the more abundant mucopolysaccharides which facilitate the gliding of muscle and other fibers upon each other in the tissues just as they ease the friction between joint surfaces. The increased stiffness which these patients experience when cold is probably the exaggeration of a common phenomenon. Cooling a limb produces a greater fall in temperature within the joints than in skin or muscle and the viscosity of synovial fluid is increased by lowering the temperature[56]. It is understandable that increased resistance to movement might further embarrass an already weak muscle whose fibers are "sticking."

Some fibrosis of joint capsules is common in severe osteoarthritis and clearly may restrict movement. Fibrosis is particularly common in the hip and is usually most marked in the inferior part of the capsule. This part becomes taut near the limits of extension, abduction and internal rotation of the femur and, because of the partly spiral arrangement of capsular fibers, extension tends to force the femoral head up into the acetabulum[137], locking the joint for standing. Lloyd Roberts[81] has pointed out the importance of scarring and shortening of the

inferior part of the capsule in causing loss of full range of these movements, which is often an early sign of hip disease.

The joint capsule is a sensitive structure, to a much greater degree than cartilage or synovial membrane[71]. Traction upon the shortened capsule may therefore be expected to cause pain and muscle spasm, thus perpetuating and increasing deformity and restriction of motion. It is interesting that radiological evidence of periosteal new bone formation may be seen on the under aspect of the femoral neck in patients with marked loss of hip motion, suggesting bony proliferation in response to traction at the insertion of the inferior joint capsule[80].

The development of capsular fibrosis is usually attributed to altered joint stresses, pressure of excrescences and the occurrence of minor hemorrhages from awkward use or trauma; however, deposits of hemosiderin are infrequent[81]. Cartilaginous fragments, which are not metaplastic and are presumably derived from destruction of joint surface, are frequently found in the synovial membrane of osteoarthritic hip joints, particularly in the inferior part, to which they might settle by gravity. These fragments are very slowly digested and excite fibrosis in adjacent tissue[81]; they may well be an important cause of scarring in the hip capsule.

The deep pain which may occur is usually associated with advanced joint destruction and is probably vascular in origin[137]. It appears clinically to resemble the bone pain of Paget's disease, another condition associated with increased vascularity and trabecular remodeling. Pain at night may well represent a reactive hyperemia and congestion in bone capsule or periosteum after the day's use; all these tissues are highly sensitive to changes in tension. Repeated transmission of intra-articular pressure into a cyst communicating with the joint cavity might well excite hyperemia and the wall of such cyst is, in fact, highly vascular[137]. Equally the stretching of scarred capsule or the occurrence of trabecular fracture might be expected to produce hyperemia and pain both during prolonged use and afterwards. No benefit follows muscle transplanting into the femoral neck to try and increase the blood supply[89], but the pain of osteoarthritis is sometimes relieved by radiotherapy[34], which leads to vascular obliteration, or by simple elevation of the affected hip joint to aid venous drainage[39]. Thus, although the precise importance of several possible sites of origin of pain remains uncertain, the idea that the pain is fundamentally vascular in origin has much to commend it.

Diagnostic Features. The well known clinical and radiological features seldom cause diagnostic difficulty when osteoarthritis is localized, though as it is so common there may be occasional uncertainty whether it is the cause of a patient's symptoms or simply coincidental and unrelated. In the generalized form[70] confusion with rheumatoid arthritis is possible, especially when the proximal interphalangeal joints of the fingers are involved and the sedimentation rate is raised. However, the pattern of joint involvement in the hand in osteoarthritis differs from rheumatoid arthritis, the brunt of the damage falling on distal interphalangeal and the first carpometacarpal joints, proximal interphalangeal joints being affected to a lesser degree and metacarpo-phalangeals seldom. These features, with the lack of constitutional disturbance, the negative agglutination tests for rheumatoid arthritis and the results of careful radiography should enable accurate diagnosis.

Radiological features do not need any comment here. The establishment of standard grades and criteria for diagnosis, and the analysis of the variability resulting from the reading of radiographs by different observers[67] represent an important advance in facilitating the comparison of population surveys and of the results of other investigations obtained in different centers.

Thanks to radiology the diagnosis of osteoarthritis is seldom completely overlooked. The opposite error, of too readily attributing symptoms to osteoarthritis on the basis of roentgenographic evidence, is a constant hazard, for roentgenographic changes are usual in middle life and beyond, and in most instances are not associated with any notable symptoms. It is always the clinician's responsibility to decide whether osteoarthritis, as revealed by Roentgen-ray, is the cause of the patient's troubles or simply a chance, unrelated finding.

TREATMENT. *General.* True prevention of the disease is at present impossible and must await the discovery of a means of arresting the degradation of articular cartilage and of a method for the diagnosis of the earliest changes. However, much may be done by trying to eradicate predisposing factors, such as obesity, and by the meticulous care of the deformities, injuries and diseases of bones and joints occurring in younger patients.

The management of patients with established osteoarthritis has been frequently reviewed[3,15,64,83,84,97,133]. The basic principles, on which most authors agree, but which are often disregarded in practice, are the avoidance of predisposing or aggravating factors and the correct use of rest and of physical treatment.

In preventing further deterioration of an affected joint (and nearly all weight bearing joints are affected pathologically in middle life) the avoidance of excessive or needless use is cardinal. In this connection the designing of suitable work places and homes to spare the elderly joint merits much greater attention[50], for the industrial implications of the incapacity due to severe osteoarthritis in experienced senior workers are considerable[47]. The suitability of senior members of the Armed Forces for active service may call for nice judgment when it is recalled that 62% of patients treated for osteoarthritis at hospitals in the Mediterranean theatre had to be evacuated[104].

The evils of poor posture and obesity, attested by clinical experience, population surveys[68] and experiment[107] are still too little appreciated by the lay public, whose ideal of civilization too often appears to be "Musculi parvi in corpore flacco." The physician may encourage ladies to lose weight by recalling that low calorie diets retard aging[107]. My colleague, Dr. Maridel Saunders, suggests prone lying at mealtimes as an aid in reducing both weight and flexion deformity.

The value of rest is considerable, particularly when secondary inflammation is present; the virtues of rest and restriction of activity have been personally attested[13]. But rest must be planned and interspersed with correct physiotherapy; unsupervised immobility is hazardous[49]. Physiotherapy aims to relieve pain, retain movement, strengthen muscles and correct posture[64]. The first two aims may be achieved by gentle heat, wet or dry, and by active assisted exercises, often best performed with gravity largely eliminated by slings or by doing the exercises in water. Resisted exercises with a minimum of joint motion are needed to counter the muscle weakness of advancing years and diminished use.

These exercises also improve the awareness of muscle and joint action, which may have been impaired by slackness of muscles and altered joint mechanics, and thus reduce the tendency to further joint damage from unexpected or abnormal stresses.

Drugs. For the relief of the moderate degree of pain and stiffness commonly encountered in osteoarthritis, salicylates remain unexcelled. Their tendency to cause gastrointestinal bleeding is rarely a practical problem. It is said that severe nocturnal hip pain may be relieved by sleeping with the foot of the bed elevated, soft tissue congestion and edema being thereby reduced[39].

Many other agents have been advocated but most of the reports lack control observations or clear definitions of the criteria used in assessing improvement. Sidel and Abrams[105], in a controlled study of a streptococcal vaccine, found that 56 out of 64 osteoarthritic patients (86%) showed definite symptomatic improvement when given weekly subcutaneous injections of saline. They commented wisely on the important role played in the relief of symptoms by the physician's interest in the patient" especially when the interest takes a tangible form such as frequent needle injections." Other workers[136] found that 59% of 182 osteoarthritic patients obtained some relief from lactose pills (66% in mild cases), and of those who relapsed or did not respond, 57% showed improvement after saline injections. The present review will, therefore, ignore reports of uncontrolled trials of exotic remedies; if their authors had recalled the inability of adult articular cartilage to regenerate, some of the effort spent in such trials might have been devoted to more fundamental and ultimately far more rewarding studies.

In the occasional patient with severe pain, refractory to simpler measures, phenylbutazone may be effective in large doses[30], but this is not invariable[97]. A pragmatic trial may thus be justified in the presence of severe symptoms but not otherwise.

The systemic use of steroids does not give much, if any more relief than aspirin[35,146] and is thus unjustified[14,43,45], particularly in these older patients who are already prone to osteoporosis. By local injection, hydrocortisone may relieve symptoms[43,53] though the extent of its superiority over placebo injections has not been established[90]. A prolonged series of injections may accelerate joint destruction[23], just as in rheumatoid arthritis[24]. Hydrocortisone is probably best reserved for the relief of pain caused by inflammation secondary to joint trauma[150].

Proof of the efficacy of other intra-articular injections is lacking. The addition of lactic acid injections[144] to simple physiotherapy did not improve the results of treatment[76]. In controlled trials lactic acid was not significantly more effective than procaine or saline[33,90] or than a sterile needle prick[33] in relieving pain or in increasing motion in osteoarthritis of the hips or knees. All the procedures gave some relief and it appears that a placebo effect (Sidel's "tangible interest") is responsible for much of the benefit after intra-articular injections. The relief is real, albeit often temporary, and may encourage the patient to cooperate better with the more useful procedures of physiotherapy. However, since the various intra-articular injections are given essentially for symptomatic relief and none has been proved to exert any favorable effect whatsoever on the pathological process, it may be prudent not to run the risks involved in introducing any foreign substance into joint cavities before ascertaining whether a simple ostentatious needle

prick and mock injection[90] into the subcutaneous tissues over the joint will give an adequate response.

Radiotherapy. In a controlled trial[34] local radiotherapy yielded symptomatic relief in some cases and may justifiably be tried in these older patients with severe refractory symptoms without the anxiety about the later development of leukemia which now bedevils the treatment of spondylitis in young men. However, the relief is by no means invariable or lasting[92] and with lower doses is negligible[32].

Surgery. In addition to their role in preventing osteoarthritis by meticulous care of bone and joint abnormalities in youth, orthopedic surgeons have much to contribute to the management of the established condition in guiding efforts to retain good posture, prevent deformity and improve the mechanisms of motion. In the more advanced cases who are candidates for operative intervention, the selection for operation of only those patients in reasonable physical and emotional health is essential; in an otherwise healthy patient even arthrodesis of the hip may be a relatively slight disability[143]. Assessment of the various operative procedures is beyond the scope of this article and several general reviews are available, mostly pertaining to the hip[28,88,145]. Other papers relate the results of arthrodesis[143],osteotomy[1,20] and a combination of the two procedures[135]. Osteotomy has been alleged to allow regrowth of cartilage on the femoral head[137] but the observed increase of "joint space" is more probably due to the different angles at which postoperative radiographs are inevitably taken because of the adducted position of the upper fragment[2]. The merits and disadvantages of arthroplasties and of various prostheses have also been discussed[73] and possible methods for the surgical relief of other affected joints have been summarized[5].

Because articular cartilage is nourished by synovial fluid it can survive transplantation from one joint to another in the same patient. The suggestion that destroyed cartilage in an important joint may be successfully replaced by a viable graft taken from a relatively "expendable" joint[44], points to a possible line for future progress and represents a hopeful note on which to end this review.

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NEUROLOGY AND PSYCHIATRY

UNDER THE CHARGE OF

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THE SCHIZOPHRENIAS, 1959: A REVIEW

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THE discussions of schizophrenic diseases during the last year have had mostly to do with drug treatment of the diseases, if simple volume of material be used as the criterion. Other areas of interest: etiology, treatment methods other than pharmacologic, personal relationships between schizophrenic patients and other significant persons in their lives, have continued very actively to be represented in the literature. These discussions are well reviewed in psychiatric journals (Alanen[2], Blèuler[6]).

In terms of observable clinical effects, the drugs fall into three classes: hallucinogens, those which produce marked alteration of the psychological status of normal persons, usually with visual hallucinations, disorientation, altered perception of time and body image; tranquilizers, which for the most part reduce anxiety and psychotic symptoms by action antagonistic to serotonin in the brain stem; and energizers, some but not all of which are mono-amine oxidase inhibitors.

The hallucinogenic drugs, lysergic acid diethylamide (LSD), mescaline and others have been considered to produce a "model psychosis," more easily available for study. Although there is now a clear consensus, beginning with the first reports (Stoll[30]) that LSD produces a delirious state which is not schizophrenia, and not very similar to it, still the mistake continues to be made to assume that they are the same.

There exists now another technique which produces a very disturbed psychological state, that is, sensory deprivation (Lilly[17]). Here again the changes produced are not the symptoms and signs of schizophrenia, but we find Rosenzweig[23] again speaking of 'an experimental model of the schizophrenic syndrome."

On a purely psychological level, the phenomenology of these delirious states is now fairly well described, both objectively and subjectively (Macdonald and Galvin[18]). One area which has been discussed (Stoll[31]) but not, so far as I know, investigated, is the methods available to the delirious individual to reduce or control the abnormal experiences which he is suffering. We have long known that vision helps moderate the hallucinations of delirium tremens; and that these patients become worse in the dark. Individual

patients, intoxicated with LSD, report maneuvers by which, apparently, visual hallucinations can be interrupted. Among these is fixing attention on some simple, concrete, geometric figure. Theoretically and practically, a description of the ego attempt, whether succeeding or failing, to master these artificially induced deliria would be most useful.

The tranquilizing drugs are being much written about. They fall into several major chemical classes: the Rauwolfia derivatives; the phenothiazine compounds; and a third group of differing compounds including meprobamate and hydroxyzine.

This literature is difficult to evaluate for many reasons. About 16 drugs are represented by many publications each, during the last year. Many of them report impressionistically on small numbers of patients. Even the best of them have to deal with the major problems of homogeneity of the group being treated, control, and operational criteria for evaluation of results. It may be significant that so few papers report disappointing results (Feldman[9]).

Benjamin's[5] discussion of biological research is an important document pertinent to these and other problems.

It is widely reported and believed that tranquilizing drugs are useful as an aid to psychotherapy. This reviewer seriously doubts that claim. There can be no doubt that some patients are not amenable to psychotherapy because they are too excited or disturbed, but reducing the excitement does not necessarily bring about the conditions for psychotherapy. Indeed, some patients are relatively so free of painful symptomatology while taking tranquilizers, that they deliberately choose to continue a symptomatic treatment by drugs, rather than a truly therapeutic regimen. There are many opinions to the effect that the tranquilizers are at best symptomatic drugs (Cobb). Others are impressed by the usefulness of drugs as adjuncts to psychotherapy (Hoch[13]).

The term "psychic energizers" is much newer than the use of this kind of drug. Caffeine, ephedrine, benzedrine and other central nervous system stimulants somehow never earned so esoteric a title.

Most of the new energizers are not directly pertinent to the treatment of the schizophrenias, unless the total clinical picture includes, as is not infrequent, both schizophrenic and depressive features.

One drug, trifluoperazine, has been recommended for the reactivation of patients with chronic and severe social withdrawal, and who fit into one of the schizophrenic categories. This drug is not yet adequately evaluated.

A very basic opinion of schizophrenia expressed in 1911 by Professor Eugen Bleuler when he gave us the phrase "The Group of Schizophrenias," is now being discussed with renewed interest. There is widespread feeling (Rosen[21]) that the nosologic system at present in use does not reflect the knowable reality. First, patients change from one schizophrenic subgroup (catatonic, paranoid, and others) to another, sometimes within a short time. Further, patients may support a diagnosis of depression at one time, and schizophrenia at another. I do not believe that careless diagnosis is the explanation for all such cases. Certainly, an arbitrarily unitary view of a multiple variable problem can serve as a major hindrance to improved understanding.

The theories of etiology currently being discussed can be divided into several groups. It is again worthy of note that most investigators work in only one of these areas. Limitations of specialized skills can hardly explain this phenomenon. Voices have been raised in protest (Malamud and Overholser[19]).

The major classes of etiology now being considered are hereditary, physiologic and environmental.

On the subject of an hereditary component in the schizophrenias, Kallman[14] in this country, and Slater[27] in England, continue to be major contributors. The general field is discussed by Rosenthal[22] in a paper which supports "biological heterogeneity of schizophrenia." Here is again repeated, on the basis of identical twin studies, the well-supported conclusion that genetic factors do affect the incidence, course and outcome of the schizophrenic disease, although there is not agreement on exact patterns.

Kraepelin[16], in the spirit of his times, felt that dementia praecox was caused by an anatomical or physiological abnormality of the brain. This opinion continues to be held, at least for physiology, although the general position is that the several physiological deviations from norms that have been described must be considered constant concomitants, and have not been proven to be causes or effects. Among these have been oxygen metabolism (Himwich[12]); adrenal function (Pincus and Hoagland[20]); ceruloplasmin (Akerfeldt[1]); taraxein, apparently a form of ceruloplasmin (Heath[11]); serotonin (Woolley and Shaw[34]) which compound is antagonized both by lysergic acid diethylamide, the "hallucinogen," and by reserpine, the "tranquilizer;" mono-amine oxidase, which destroys serotonin, and for which inhibitors are available; tryptophane metabolism, measured by excreted products (5 hydroxyindoleacetic acid) (Kopin[15]).

A good deal of the theorizing about possible physiological abnormalities now carries the implication that the abnormalities, if adequately delineated, may be correctible, either by pharmacologic or psychotherapeutic means.

The psychotherapy of the schizophrenias, with the objective of *restitutio ad integrum*, or "far-reaching improvement" (Benedetti[4]) is now well known. Very useful as a research tool, statements as to the relative success of these methods cannot yet be made. The therapy is slow and difficult, certainly not suited, in any present modification, for mass treatment.

Fromm-Reichmann, Sechehaye, Benedetti, Rosenfeld are all reporting, from time to time, activities in this field.

There is considerable opinion to the effect that the schizophrenic patient, even though he does not present the full-blown symptomatology until later in life, suffers serious developmental damage very early in life, at a time when the mother is still the paramount object in his life. Spitz has shown with dramatic clarity how catastrophic can be the effects on the child who suffers severe deprivation in infancy. Therefore much attention has been paid to the early mother-child relationship, in the attempt to show out of what kind of living experience the schizophrenias grow.

The careful and well-documented work by Alanen[2] studies the personalities and relationship of 100 mothers of schizophrenics. He demonstrates a high incidence of an attitude which he calls "schizoid pattern of interpersonal relationships," a tendency to dominate, little concern for the child's needs and powerful possessiveness. These views are very similar to another report of preliminary findings in a much smaller group of mothers (Galvin[10]). Neither of these is controlled; that is, there is no demonstration that the characteristics are limited to the mothers of schizophrenics.

Searles[24] has one of the few papers dealing with positive and loving feelings between mother and child, which feelings he thinks are present and

strong, although denied. He also makes the point, not, I believe, generally accepted, that from the very beginning of life the baby has not only the need to receive love, but also the need to give it.

In another paper Searles[25] restates the fact of the sensitivity of the schizophrenic to unconscious processes in the therapist. The surprise experience of the therapist attending this phenomenon is often strongly suggestive, if not diagnostic, of a schizophrenic disease.

Bateson and others have developed the concept of the "double bind" as an attempt to typify the usual kind of relationship between the schizophrenic and his mother. This is meant to describe a situation of dilemma, in which the child is given contradictory prohibitions of instructions, forbidden to escape, and threatened with very severe punishment. He shows possible consequences in later life of this kind of relationship.

Another study has been made by Brodey[7], on the basis of a unique research situation, involving a study of 5 families, each having a schizophrenic member, which families lived in hospital under observation for periods ranging from 6 months to 2½ years. These families may or may not be typical. It was striking that hospital staff tended to assume roles very like those of family members. Thus, a nurse might act like the mother, even being grandiose and babying the patient. There were often great tensions between family and staff. Individuals in the family managed to maintain an extreme lack of awareness of and sympathy with other family members. In this paper, too, are given repeated examples of the peculiarly close and long lasting intimacy between mother and child. Brodey uses the assumption of circular causality, that is, the family in which a schizophrenic appears, contributes to his disease and is also affected by it.

These samples, from each of the major points of view of the schizophrenias at the present time, are intended to give some overall idea of the thinking and research activity going on.

By way of summary, it is probably fair to say that "schizophrenia" represents a group of diseases which need better diagnostic delineation, probably on the bases of etiology and genesis. The etiologies may well depend upon configurations of multiple variables. Hereditary factors, neurophysiologic abnormalities and traumata in the very early mother-child relationship, may well all play parts.

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BOOK REVIEWS AND NOTICES

NEGROES AND MEDICINE. By DIETRICH C. REITZES. Pp. 400. Cambridge, Mass.: Harvard University Press, 1958. Price, $7.00.

THE purported raison de'être is to discuss the integration of Negroes in medicine, and to provide data on the general problem of medical services for and by Negroes, since they constitute 10% of the total population but only 2.2% of all physicians.

As a sociological study in depth, it has succeeded admirably. The problem is attacked from several angles: training of prospective physicians (Negro undergraduates do proportionately poorer on the MCAT test and apply at fewer universities, quantitatively limiting their chances for acceptance); integration of Negro physicians in the community (depending on population, geographic area and economic mores, that is, many Negro middle and lower class patients in Philadelphia preferred or were accustomed to clinic care rather than a private physician, regardless of his race); cooperation between Negro and white physicians (usually adequate since the Negro physician general practitioner did not feel that the white specialist was competing with him. However, between Negro general practitioner and Negro specialist, the prevailing attitude as expressed was "dog eat dog."

The book is a careful blend of statistics and opinions, with the latter clearly labeled as such, and the tables and appendices simply constructed and pertinent. The conclusions, if they may rightly be called that, are offered with diffidence, and suggest that the laissez-faire course is not adequate toward increasing the number of Negroes in medicine, and special action is still needed, being careful not to "reward mediocrity;" an attitude empirical as it is prevalant. R.B.

STRABISMES: HETEROPHORIES, PARALYSIES OCULO-MOTRICES. (LES DÉSÉQUILIBRES OCULO-MOTEURS EN CLINIQUE). By RENÉ HUGONNIER, Ophtalmologiste des hopitaux de Lyon. Pp. 748; 230 ills. Paris: Masson et Cie. 1959. Price, 8.500 fr.

THE beginning student of Ophthalmology, if he is at all familiar with the French language, will find this textbook a pleasant key to the mysteries of oculomotor dysfunctions. Writing primarily for the novice and for the student of orthoptics, the author has presented a concise and lucid explanation of the complex mechanisms of strabismus. The book deals with elementary principles and carefully avoids highly controversial opinions. The reader will find, however, a complete and understandable discussion of all important phases of the various forms of strabismus. A helpful glossary of terms is included early in the text. Each section is carefully arranged in logical sequence with numerous subtitles designed to permit easy reference, and the text is concluded with a helpful list of aphorisms summarizing the most important principles in diagnosis and treatment. W.F.

NEUROSURGERY DURING WORLD WAR II. VOLUME I. Prepared and published under under the direction of Major General S. B. Hayes, Surgeon General United States Army. Editor-in-Chief, COLONEL JOHN BOYD COATES, JR., Medical Corp. Editors for Neurosurgery, R. GLEN SPURLING, M.D. AND BARNES WOODHALL, M.D. Associate Editor, ELIZABETH M. MCFETRIDGE, M.A. Pp. 466; 130 ills. Washington, D.C.: Surgeon General Department of the Army, 1958. Price, $5.00.

THIS volume deals with administrative considerations of Neurosurgery and with head injuries. The second will cover injuries to the spine, rupture of the intervertebral disc and peripheral nerve injuries.

The contents are divided into Part I: Administrative Considerations in Neurosurgery and Part II: The Management of Head Injuries. This consists of 13 chapters and includes the management of acute cranio-cerebral injuries due to missiles; infections; cerebral injuries due to explosion waves; cranioplasty; post-traumatic epilepsy; speech disorders; and clinical pathologic aspects of fatal missile-caused cranio-cerebral injuries.

The appendixes include: (1) Course of Instruction in Neurosurgery, (2) Extracts from the manual of therapy, European theater of operations, (3) Special history and examination forms and, (4) Battle wounds and battle injuries of the head and brain.

This volume is fascinating reading for those interested in the history of Neurosurgery in the U.S. Army. It contains a wealth of information on the management of trau-

matic injuries of the skull and brain and should be indispensable to the Neurosurgical resident, the Neurosurgeon, the general surgeon treating head trauma, to the Oto-Laryngologist who occasionally has to treat sinus injuries, to the speech therapist and to the Neurologist who frequently sees these patients.

M.S.

CHILDBEARING BEFORE AND AFTER 35. By ADRIEN BLEYER, M.D. Pp. 109; 14 ills. New York, Washington, Chicago, Hollywood: Vantage Press, 1958. Price, $2.95.

ALTHOUGH there is a considerable amount of information available in the literature documenting the dangers of pregnancy during the late years of the reproductive period few physicians are aware of how important age is in the development of normal offspring. Bleyer has consolidated the available studies into a small volume dedicated to the thesis that the healthiest infants are born to mothers between the ages of 20 and 30 and the information presented substantiates his opinion without question.

Not only are prospective mothers over age 35 subject to an increased personal risk from various medical diseases which appear at this period of life, but their infants are less likely to develop normally and to survive. The incidence of congenital defects such as mongolism, mental deficiency, achondroplasia, heart lesions and others increases steadily with advancing maternal age.

The material is documented by numerous references to scientific papers and illustrated by charts, graphs and tables taken from the publications. Although the references would be a bit difficult to collect because they are scattered throughout the text, they are complete.

This book, which is written in a simple, chatty style, should serve a useful purpose for both physician and layman. It will make the former think a long time before glibly approving a young couple's postponing childbearing for any period of time, particularly if they are in their late twenties. If it were read by all young newly married couples it could be of great importance in helping them plan a reproductive schedule.

J.W.

THE POSTNATAL DEVELOPMENT OF THE HUMAN CEREBRAL CORTEX. By J. LEROY CONEL, Research Associate in Pathology, Children's Medical Center; Professor of Anatomy, Emeritus, Boston University School of Medicine, Boston, Massachusetts. Pp. 309; 201 ills. Cambridge, Mass.: Harvard University Press, 1959. Price, $12.50.

THIS large book is Volume 6 of a study of the postnatal development of the human cerebral cortex. This volume deals with the cortex of the 24-month infant. Six brains were used for this investigation. They were obtained at autopsies performed in the department of pathology in the Children's Medical Center in Boston.

Blocks of tissue were cut from the same areas of the cortex and sections were prepared in the same manner as in all of the earlier stages of development described by the author in Volumes 1 to 5 inclusive in this series of monographs.

The contents are divided into a study of the cortex of the frontal, parietal, occipital, and temporal lobes. A separate section is devoted to the insular lobe and to the rhinencephalon. Section on comments, tables, and bibliography completes the monograph.

The data is profusely illustrated by 201 photomicrographs and drawings of sections of the cerebral cortex from the various lobes of the brain. All the photographs are magnified by 100 diameters. The drawings of neurons are made with a camera lucida from the Golgi-Cox sections and are magnified by 250 diameters. Representative cells are drawn in each layer of the cortex.

This volume represents an exhaustive and careful presentation of the cyto-architectonics of the cortex of a 24-month infant. It is hoped that this work will give a better foundation for studying the architectural structure of the brain as it developes its adult stage.

This volume should be of interest to anatomists, neuropathologists, and neurophysiologists and to anyone interested in the development of a human cerebral cortex.

M.S.

BONE TUMORS. By LOUIS LICHTENSTEIN, M.D., Veterans Administration Center Hospital, Los Angeles. 2nd ed. Pp. 402; 220 ills. St. Louis: C. V. Mosby Co., 1959. Price, $12.00.

OVER the past 7 years, Dr. Lichtenstein's *Bone Tumors* has become the standard reference for pathologists, orthopedists and radiologists. The usual "second edition" changes are found in this volume and the book is substantially strengthened by them. A new chapter on neoplasms of the periosteum has been added. Not everyone will agree with his concept of juxtacortical ossifi-

cation and its frequency in relation to neoplasms of this area. His classification of these tumors seems a bit complex and nebulous because apparently he does not recognize as an entity a tumor of mixed type which has been called parosteal sarcoma by some. However, he admits that his stand in the past may have been somewhat too conservative and states that the answers to our diagnostic problems in this area lie in the correct interpretation by the microscopist and with this no one would disagree.

For a concise, authoritative and intelligent review of the subject, Bone Tumors cannot be beaten and moreover the succinct and lucid writing and case matched illustrations makes it a pleasure to read. E.A.

HANDBOOK OF RESPIRATION. Analysis and Compilation by PHILIP L. ALTMAN, JOHN F. GIBSON, JR., CHARLES C. WANG. National Academy of Sciences, National Research Council. Pp. 392; illustrated. Philadelphia: W. B. Saunders Co., 1958. Price, $7.50.

THIS handbook is a collection of most valuable data which should be in the possession of every person who is interested in respiration. Many data have been brought together (by competent authorities) which cannot be easily found under one cover anywhere else. It is detailed, reliable and covers the area thoroughly. The references alone would make this a desirable book. These are very extensive and modern. M.O.

Les Hémoptysies Trachéo-Bronchiques. BY ANDRÉ MEYER AND JACQUES CHRETIEN. Pp. 158; 23 ills. Paris: Masson et Cie, 1958. Price, 2000 francs.

IN this monograph the authors have reviewed in a very thorough manner, their experiences with 717 patients with hemoptysis. In 454 of these patients the bleeding was coming from the lungs. In 135 the bleeding came from the tracheobronchial tree. In 128 instances it was not possible to ascertain the site of bleeding. The common causes for bleeding from the tracheobronchial tree were cancer and bronchiectasis, while from the lung, tuberculosis was the chief offender. A critical study was made, not only of the clinical material, but also of the bronchoscopic and pathologic material. An analytical study was also made of the rarer causes of hemoptysis. The authors cover the subject well and document their findings. Therapy is considered briefly.

J.J.

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